Comment by comment responses to reviews, together with a document where track changes were used to show all changes between the first submitted version and the current submission.

Anonymous Referee #1 Received and published: 10 October 2016

Comment: In this paper, authors try to link the observed chemical properties in peat profiles (C, N, and their isotopic composition) with current and historical biogeochemical processes over the several thousand years of peat development. Using the multiple regression analysis, they conclude that the peat profiles were controlled by the Suess effect, vegetation, hydrology, and fractionation involving C-N chemical bongs. Their approach is interesting and the information can potentially help better understand biogeochemical processes associated with peatland development.
My biggest concern is that the conclusions are drawn heavily from the results of multiple regression analysis. If I understand the Appendix 1 correctly, it appears that 238 C1 data points were regressed for 43 parameters. If this is correct, then there may be an overfitting problem, and some of the significance found in the analysis may be misleading. Combining plots and soil depth by functional groups (e.g., “near-lagg plots” vs. “away-from-lagg plots”, “modern vs. old soil horizon”, or differences in REDOX status) can reduce the number of parameters.
Response: We consulted with a statistician at UNH about this issue and he recommended transforming depth into a continuous variable, which we have now done. To account for the obvious non-linearity of the isotopic responses to depth, he also recommended including the depth, the square of depth, and the cube of depth as separate parameters (that is, a cubic transformation). This reduced the depth to three terms. Plots remained as nominal variables, but we now used a stepwise approach, which parses the plots sequentially into two groups to account for maximum variability. This reduced the number of plot variables to three as well. The best-fit models based on minimum AICc values now had only 12 terms for δ^{15}N and 14 for δ^{13}C, which is a four-fold reduction. Of the parameters previously included in the regression analysis, vegetation cover (near trees or not) is no longer a significant factor for δ^{15}N and topography is no longer a significant factor for δ^{15}N, presumably because the information is now largely included in the continuous variable of depth. The methods, results, and discussion have been changed to reflect these changes in our statistical approach.

Comment: I found the earlier part of the paper to be somewhat difficult to follow. This was mainly because of the lack of site information, which was described later in the paper. I think that it would be more helpful to the readers, who are not familiar with this particular ecosystem, if important site characteristics were described earlier in the paper. These include: the relative location of upland and lagg; hydrology differences among upland, lagg, and bog; seasonal shifts in water table (if any).
Response: The site information is now moved to earlier in the paper. Figure 2 now indicates the location of the lagg versus the bog. In addition, hydrologic information about the bog and two relevant references have been added to the first section of the Methods. We indicate that the

water table can shift up to 30 cm on an annual basis and 140 cm over the course of a 50-year record.

Comment: Finally, I think that the authors need to tie their findings back to the conceptual model presented in Fig. 1. by highlighting the correspondence and discrepancy between their conceptual model and their data in the discussion.
Response: We have now added several sentences to the concluding paragraph discussing where our conceptual model captured important features of bog C and N dynamics that influenced isotopic patterns and where our conceptual model appeared to be lacking.

Specific comments:
Comment: Hummock vs. hollow is described as "topography" in some places and "microtopography" in other. I suggest using the same word throughout the paper. Given that the authors discuss upland effects in the discussion, it is a bit confusing in some places whether they mean hummock-hollow or upland-bog.
Response: microtopography has been changed to topography. We feel that for the most part the distinction between uplands and peatland topography of hummocks and hollows is clear in the manuscript. We have added the words "adjacent" to the caption of Figure 1 to further clarify this distinction.

Comment: I suggest that the authors clearly describe what was used as a reference (0 cm) soil depth. In some places, it is referenced to the hollow surface, and other places the water table.
Response: The reference (0 cm) depth is now clearly identified as the hollow surface.

Comment: P.4, L.19 How did fine roots identified to species? I don't think the root C, N, and isotope data are not presented in the paper.
Response: Fine roots were not identified to species but were identified to broad taxonomic categories of shrubs (dicots), sedges (monocots), and conifers. However, the spatial sampling of roots was not often enough to permit each of the 17 core locations to have corresponding root data, and so root data are only presented in the text, in the first paragraph of the results.

Comment: P. 6, L.11-12 For this statement, it would be nice to have C, N, and isotope information of roots and senesced leaves. Because roots are the major player for boreal soil C C2 (Clemmensen et al. 2013) and because nearly half of foliar N would be resorbed during senescence, it would make more sense to use C, N, and isotope values of roots and senesced leaves than the values of live leaves, if the data are available.
Response: There is little or no ^{15}N fractionation during resorption, so litter corresponds closely to foliar δ^{15}N. We do give isotopes on roots, but it is not given at the species level. Litter data were not available.

Comment: P.6, L.23 “very low in %N and δ15N and high in C/N” – Showing the actual values here from the citation would be helpful.
Response: The values have been added: “very low in %N (0.22%) and δ^{15}N (-3.6‰) and high in C/N (~190)”

Comment: P.8, L.17-19 It sounds that the water table in this bog is very close to the surface. I am not sure whether the presence of trees can create a large enough gradient in water potential that would affect 13C fractionation of photosynthesis. I would assume water potential gradient to be greater between the hummock and hollow than between locations near trees and away from trees.
Response: The reviewer may very well be correct. As we worked on the revisions, we found a paper where δ^{13}C in Sphagnum was compared against water table depth, with a negative correlation and a slope of 0.04‰ per cm (Loader et al. 2016). Based on our coefficient of -0.12‰ for the tree factor, a 6 cm difference in depth to water table between treed and untreed areas would explain the difference. (Loader, N., Street-Perrott, F., Mauquoy, D., Roland, T., van Bellen, S., Daley, T., Davies, D., Hughes, P., Pancotto, V., and Young, G., 2016: Measurements of hydrogen, oxygen and carbon isotope variability in Sphagnum moss along a micro-topographical gradient in a southern Patagonian peatland. *Journal of Quaternary Science,* 31(4): 426-435.)

Comment: P.9, L.2-3 Please specify which N-loss pathways are discussed here; via DON leaching or denitrification, or both? The authors mentioned earlier that DON lost from wooded upland was more 15N enriched. Is the term “sedge system” equivalent to the wooded upland? If denitirification losses were being concerned here, then higher rates in fens than bogs (P.9, L.11) could result in N lost from fens can be more 15N depleted than bogs.
Response: The reviewer makes a good point here and we have removed the sentence (and the explanation) in our restructuring.

Comment: Table 2. Please add the information for roots.
Response: As indicated above, all the root data are given in the first paragraph of the results, so it was not necessary to add it to a table. We did not have species-specific root data.

Comment: Fig. 1. This conceptual model and the data presented in the paper do not match in some cases. I would like to see discussions that tie their results back to this conceptual model: what are the processes responsible for the discrepancies?
Response: See response to the ‘Conceptual Model’ comment (3rd comment above).

Comment: Fig. 5-b. Could it be possible to add a rough sketch of study sites, overlaying the coefficient values on each plot? Adding the relative location of upland, bog, and lagg on the sketch would help. I went back and forth between Fig.5-b and Fig. 2, only to find that the layout of the coefficient values do not align with the plot layout (there are different number of rows, for example).

Response: In the revised statistical analyses, we have less precise spatial information on how the plots differ isotopically, and therefore discuss it less. The revised Figure 2 now includes the boundary between the lagg and the bog.

Technical Comments
Comment: P.2, L.10 “d13C of photosynthesis” – photosynthates? "
Response: Changed to “plant photosynthate”

Comment: P.2, L.26-27 Rephrase to explain what “topographic effects” are considered here.
Response: The sentence in question stated: “Differential rates of net primary production and decomposition which vary by specific vegetation and water table could also contribute to topographic effects (Moore et al., 2007; Vitt et al., 2009).”
We have deleted this sentence and removed the two references from the reference list.

Comment: P.3, L.35 “the location of future experimental plots” – future?
Response: “future” has been removed.

Comment: P.6, L.24 “seven times” – how does this frequency compare with the frequency of Sphagnum?
Response: This was buried wood. Changed to “seven times during laboratory examination of the 238 samples”.

Comment: P.7, L.4 “breaking a C:N bond will discriminate against both 13C and 15N.” – please add citations for this statement.
Response: This section has been eliminated.

Comment: Fig. 1. “methanotrophy and CO2 recapture by Sphagnum” – “recaptured”.
Response: This has been changed to “methanotrophy and subsequent CO2 recapture by Sphagnum”.

Comment: Fig. 3. The C/N axis does not look like natural-logged values.
Response: The legend was changed to reflect that C/N, rather than log C/N, is plotted.

Comment: Fig. 5. “plot locations are as given in Figure 1” – should be Figure 2.
Response: Changed.

Comment: References Some citations in the text are not included in the reference list.
Response: All text citations have now been checked against the reference list.

Comment: Literature cited in this comment: Clemmensen et al. (2013) Science, 339:1615

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-261, 2016.

Anonymous Referee #2
Received and published: 26 November 2016

General responses: The reviewer provided a thoughtful critique of the paper's deficiencies. Comments were provided in a narrative style, with not many specifics, so in some cases responding adequately was challenging.

Comment: From the introduction as written, I do not 1) understand clearly the motivation to do this study (what real gap does it address?), 2) the reason for using this particular study location (aside from the fact that other stuff will be going on there), and 3) what the authors hope this paper will add to science. The authors bring up a few topics that might be useful to test, but none are presented in a way that suggests apparent urgency or immediate / profound scientific consequence.

Response: In the rewritten introduction, we lay out where we feel current interpretations of C and N isotope patterns in peatlands have been inadequate and the therefore how our approach provides additional nuance to interpreting such patterns. This study location was used because the history is well-known through study of a nearby bog and because the multiple cores taken provided a good opportunity to actually examine spatial effects and have some statistical power to examine multiple factors influencing core isotopes, unlike in the majority of previous investigations of peatland isotopes.

Comment:
In addition to the introduction's diffuse content, the writing style doesn't allow the messages to get across effectively. For one thing, the intro text contains many long sentences. The leadout sentence is an excellent example, failing to provide a hook, despite use of "imperative" as one of its 50+ words. In some sentences, words like "peat" are repeated 3 times, which could be avoided with some restructuring.

Response: We have rewritten the paper to make it easier to follow. "imperative" (somewhat hyperbole in its effect) has been replaced with "necessary". We have pruned words where possible, particularly where the same word (like 'peat') is used multiple times in a sentence.

Comment: The authors also use terminology I've never heard used before (despite decent familiarity with the topic). Not only were the terms not explained, but some were used uniquely in the introduction. If they aren't used throughout the text, one might conclude that they are superfluous - with the net effect being reader frustration if not of immediate familiarity.

Response: We are unfortunately not sure what terminology is referred to here.

Comment: Why send the reader to find a 1980s paper in order to follow the narrative?

Response: We assume this refers to the Clymo 1984 paper. This has been updated with a more recent paper (Belyea and Malmer 2004).

Comment: There is also mention of "our" conceptual model, but it’s not clear whether the authors are referring to a shared community understanding/model, or a model that they’ve discussed before in papers they have authored, or a model they are proposing for this site.

Response: We are referring to the conceptual model given in Figure 1, which was our working model for the potential factors that would influence isotopic patterns in the peat profile. We have added reference to Figure 1.

Comment: Lastly, there are a large number of goals / objectives / hypotheses, for C:N, and isotopic signatures, jumbled together in a long paragraph that sets out a scope of work. Why do the authors present only vague exploratory goals for C:N but highly specific hypothesis for isotopic patterns?

Response: The focus of the paper was on using C and N isotope patterns as a tool to better understand the processes that would affect C and N cycling in peatlands over long timescales (100s-1000s years), and specifically in peat cores. Thus, specific hypotheses for isotopic patterns were appropriate for our paper goals. Other information (like elemental concentrations and C:N ratios) could of course also provide insights, but was not the primary focus of the paper.

Comment: Which of the hypotheses are actually testable (with some definitive result)?

Response: From the perspective of having worked through the paper and the results, we would claim that hypotheses 1-3 are testable, and hypotheses 4-5 proved to be not testable in that too many competing mechanisms were available to produce definitive interpretations. We have rewritten the hypotheses now so that they are topics we can actually address.

Comment: From this introduction as written, it is difficult to figure out intent or novelty. I had to read forward into the discussion in order to understand the content a bit better. But after reading to the end, I’m unsure of motive. The second last sentence of the manuscript gives what seems to be the strongest and loftiest motivation for the study - which is to populate long-timescale (100s - 1000s years) successional models of C and N evolution, in order to test and improve our understanding of isotopic dynamics in such systems. The other motivation seems to be that the study represents baseline conditions prior to a manipulation experiment, which is certainly weaker. If that second motive were true, would it reduce this study to a pre-experimental mapping of variables? Would it be research in that hypotheses are actually being tested? I think the manuscript aims to be both, but the context and importance to both aims must be clarified. Unfortunately the authors must re-write the introduction to better articulate intent, content, and novelty.

Response: The introduction has been rewritten to better articulate our goals of exploring what isotopic patterns in peat profiles can tell us, and what limits our ability to interpret those patterns. The study certainly represents baseline conditions prior to the manipulative experiment, but that does not mean that we regard our study solely in this context. The discussion has shifted now to focus more on what we think our data are saying about the system, and less about what it did not show (for example, evidence of methanogenesis).

Comment: Despite the difficult introduction, the study itself is straightforward. A bunch of variables were collected, and analyzed by regression analysis, in order to explore changes in C and N cycles during 10,000 years of succession. In large part the C and N variations through time were explored using isotopic indicators.
The results and discussion sections are very detailed, and quite a bit clearer. Within the results and discussion, the authors interweave history (bog changes true time) and process (causal factors). It might create a less dilute narrative if these were pulled apart to some extent. Right now some sections read like a time by time (depth by depth) exploration of process. That's hard for a reader to parse. It might be better to present the history for a specific variable, and then summarize the driving processes.
In the manuscript, the whole successional question could be better partitioned from the rest because it's a sub-plot in its own. There is not a whole lot of restructuring here, but some would be useful.

Response: This was a challenge in this study, that at any time point (depth), multiple processes could be influencing a block of peat. And of course, the peat we measured reflected not only initial conditions of climate and vegetation, but also the subsequent modifications of that block of peat by numerous factors. We have done some restructuring of the discussion to address the reviewer's concerns, where but we are not sure it is feasible to restructure it in the discrete manner suggested. The discussion of isotopic patterns through time has now been separated into N dynamics and C dynamics.

Comment: I would suggest the authors use the past tense for all matters relating to results and discussion. "Isotopic patterns with depth reflect climate and vegetation", it's markedly different than saying "Isotopic patterns with depth reflected climate and vegetation". While only two letters differ, he meaning is altogether different. The first proclamation is sweeping and universal, applying at once to this system and all similar systems. The second is technically correct and measured, articulating the fact that at this place, and at the time we did our study, our data led us to believe x. While many authors can use the present tense effectively, in this case I think the past tense would work better - particularly since the study is not definitive because of interactive variables. The authors should exercise caution in their statements.

Response: We agree with the reviewer and the results and discussion have been rewritten to largely use the past tense.

Comment: The titles of the discussion sections are useful in that they articulate the main message. This approach is stylistically modern. But when overused it's dangerous in that these missives provide no nuance if not well conceived. From the existant titles, it would seem that this study is packed with a whole suite of equally important drivers of variation including climate, vegetation, suess effect, tree proximity, stoichiometry, etc. But they AREN'T all equally important. Or more accurately, they WEREN'T. I would suggest the authors revisit these titles to add nuance and accuracy. Which are "secondary" or "tertiary" drivers, for example? Which are simple co-related to some degree? An adjective or adverb here or there can go a long way. Which are a surprise, and which are somewhat self evident or expected even without the data?

Response: The order of the discussion sections has been redone to focus first on time-independent factors (such as shifts driven by stoichiometry) and then on time-dependent factors (the Suess effect, shifts driven by climate or peatland succession). Titles have been changed to be more revealing of their main point. For example, 4.2. “%N, %C, and C:N stoichiometry influence δ^{13}C and δ^{15}N patterns” has been changed to “%N, %C, and C:N stoichiometry primarily influenced δ^{15}N patterns”, and 4.7. “Methanogenesis and methane oxidation” has been deleted.

Comment: The scientific part of this study that gives me some cause for concern is that variables are "interactive", in the words of the authors. Another researcher with a more glasshalf-empty attitude might instead say that the variables are confounded. Because so many are related to one another, definitive conclusion is impossible. The authors mention this problem only passingly in the conclusion. But it’s important. Before the discussion, they should carve out some time to discuss this issue. It comes down to certainty.
While analytical error probably isn’t a big problem in a study like this, there is some uncertainty as to which drivers lead, and which respond. And that sort of understanding is critically important when building mechanistic models (a proposed follow up activity). The authors could provide some better forward navigation here, otherwise their results may be interpreted or used by others incorrectly or out of context.

Response: We believe that the concerns about ‘confounded’ variables have been largely dealt with in our statistical reworking of the data, as suggested by Reviewer #1. The revised discussion focuses more directly on processes that we believe affect the observed isotopic patterns. Several sections have been eliminated, specifically a rather theoretical discussion about how nitrogen isotopes and carbon isotopes are correlated, and a discussion of methanogenesis, for which there was really no strong data.

Comment: The conclusion didn’t summarize important points or really draw any conclusions from the work. This section could be strengthened.

Response: Our revised concluding paragraph more clearly contrasts what we learned against the conceptual model that guided our investigations.

Comment: Overall, the study contains good data and is scientifically valid. The study is not highly novel it doesn’t seem, but there could be aspects of novelty that simply aren’t communicated well.

Response: The novelty of the study is probably the detailed data set of C and N isotope patterns in a well-studied peatland. Our statistical approach to understanding these isotopic patterns was also novel, we believe, although we also concluded that the multiple processes that could influence the isotopic patterns limited our ability to pin isotopic patterns to unique factors.

Comment: The datasets and findings will likely be of use to the community of peatland researchers. Some discussion around the topic of uncertainty is warranted, resulting from the problem of confounded variables.

Response: The statistical approach has been redone in response to comments of Reviewer #1 and the issue of confounded variable has been reduced by greatly decreasing the number of fitted variables (from ~40+ to 10 and 13).

Comment: The manuscript needs work to improve 1) clarity, 2) accuracy, and 3) narrative.
Response: We believe that the revised manuscript has improved in these three areas.

14. Feb. 2017

Long-term Carbon and Nitrogen Dynamics at SPRUCE Revealed through Stable Isotopes in Peat Profiles

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Abstract. ~~We~~Peatlands encode information about past vegetation dynamics, climate, and microbial processes. Here, we used δ^{15}N and δ^{13}C patterns from 16 peat depth profiles to ~~interpret changes in C and N cycling in~~deduce how the biogeochemistry of the Marcell S1 forested bog in northern Minnesota responded to environmental and vegetation change over the past ~10,000 years. In multiple regression analyses, δ^{15}N and δ^{13}C correlated strongly with depth, plot location, C/N, %N, and each other. ~~Continuous variables in the regression model mainly reflected ^{13}C~~Correlations with %N, %C, C/N, and ~~^{15}N fractionation accompanying~~the other isotope accounted for 80% of variance for δ^{15}N and 38% of variance for δ^{13}C, reflecting N and C losses~~, with an estimated 40% of fractionations involving C-N bonds.~~. In contrast, ~~nominal variables such as plot,~~correlations with depth~~,~~ and ~~vegetation cover~~topography (hummock or hollow) reflected peatland successional history and climate. Higher δ^{15}N ~~and lower δ^{13}C~~ in plots closer to uplands may reflect ~~distinct hydrology~~upland-derived DON inputs and accompanying shifts in~~ C and~~ N dynamics in the lagg drainage area surrounding the bog. The Suess effect (declining $\delta^{13}CO_2$ since the Industrial Revolution) ~~and aerobic decomposition~~ lowered δ^{13}C in recent surficial samples. ~~A decrease of 1‰ in the depth coefficient for~~High δ^{15}N from -35 cm to -~~25~~55 cm probably indicated the depth of ectomycorrhizal activity after tree colonization of the peatland~~. Low δ^{13}C~~ over the last 400 years, as confirmed by the occasional presence of wood down to -35 cm depth. High δ^{13}C at ~4000

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years BP (-65 cm to -105 cm) could reflect a transition at that time to slower rates of peat accumulation, when ^{13}C discrimination during peat decomposition may increase in importance. Low δ^{13}C and high δ^{15}N at -213 cm and -225 cm (~8500 years BP) corresponded to a warm period during a sedge-dominated rich fen stage. The above processes appear to be the primary drivers of the observed isotopic patterns, whereas ~~higher δ^{13}C thereafter reflected subsequent cooling. Because of multiple potential mechanisms influencing δ^{13}C,~~ there was no clear evidence for ~~the influence of methanogenesis or~~ methane ~~oxidation on bulk~~dynamics influencing δ^{13}C~~.~~ patterns.

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1 Introduction

Carbon (C) and nitrogen (N) cycling are tightly linked (Schlesinger et al., 2011) and understanding the controls of C and N turnover in boreal peatlands is ~~imperative~~fundamental to ~~predict~~predicting whether this ecosystem will continue to function as a strong C sink or ~~change~~switch to a source of ~~C, in the forms of atmospheric~~ carbon dioxide (CO_2) and methane (CH_4~~), in the future~~) in response to environmental change. While 80-90% of C deposited in peatlands is lost via decomposition and microbial respiration in the upper aerobic layers of the acrotelm (~~Clymo, 1984~~Belyea and Malmer, 2004), the deeper anaerobic catotelm accumulates recalcitrant *Sphagnum* litter and other organic matter due to low mineral nutrient availability and water-logged conditions. Carbon loss from the catotelm can be 50% within the first 1700 years with only an additional 15% over the next 5800 years (Kuhry and Vitt, 1996), thus making peatlands an important long-term C sink. ~~Analysis of C and N in peatland cores is a potential way to determine key biochemical processes involved in organic matter burial and release.~~

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~~Cores taken through the peat profile in peatlands trace the trajectory of peatland succession and contain the biogeochemical fingerprint of shifts in climate and peatland vegetation states. For example, groundwater-fed fen peatlands and rainwater-fed bog peatlands differ in their pH, redox state, and balance of vascular plant versus *Sphagnum* abundance, with the transition from fens to bogs affecting biogeochemical processes and composition of organic matter throughout the peat profile (Vitt and Weider, 2006). Warming, drying, and increased N availability can also alter plant community composition, with concomitant effects on C and N dynamics, including enhanced production of greenhouse gases such as CO_2, CH_4, and nitrous oxide (N_2O) (Yavitt et al., 1987; Regina et al., 1996; Bergman et al., 1999; Juutinen et al., 2010).~~

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Factors influencing C and N dynamics can be investigated using stable isotope measurements because biochemical and physical reactions proceed faster with lighter isotopes (^{12}C and ^{14}N) than with heavier isotopes (^{13}C and ^{15}N). ~~As a result~~Thus, different pools and fluxes can vary in their isotopic signatures (expressed as δ^{13}C and δ^{15}N). Climate and foliar %N can also influence the δ^{13}C of plant photosynthate by determining the relative rates of stomatal flux versus fixation of CO_2 (Brooks et al., 1998; Ménot and Burns, 2001; Sparks and Ehleringer, 1997). Radiocarbon measurements (^{14}C) are also important in biogeochemical research, as they allow dating of peat profiles and linking stable isotope patterns to specific climatic periods or vegetational phases of peatland succession. ~~In peatlands, the~~The dominant factors influencing organic C and N turnover can be identified by characterizing isotopic signatures of specific compounds or plant components through the peat profile (Nichols et al., 2009; Gavazov et al.,

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2016), but interpreting bulk peat signatures remains challenging. Deeper peats reflect both historic vegetation as well as accumulated effects of anaerobic fermentation occurring over thousands of years. In contrast, aerobic decomposition in shallower peats alters biogeochemistry over shorter time scales.

Analysis of C and N in peatland cores is a potential way to determine key biochemical processes involved in organic matter burial and release. Cores taken through the peat profile trace the trajectory of peatland succession and contain the biogeochemical fingerprint of shifts in climate and vegetation states. For example, fens and bogs differ in the chemical and isotopic composition of the organic peat profile, reflecting differences in their pH, redox state, hydrologic inputs, and relative abundance of vascular plants compared to *Sphagnum* (Vitt and Weider, 2006). Warming, drying, and increased N availability can also alter isotopic composition through changes in plant community composition, with concomitant effects on C and N dynamics, including enhanced production of greenhouse gases such as CO_2, CH_4, and nitrous oxide (N_2O) (Yavitt et al., 1987; Regina et al., 1996; Bergman et al., 1999; Juutinen et al., 2010).

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Our conceptual model of C and N dynamics during peatland succession is shown in Figure 1. Methanogenesis, methanotrophy, refixation of methane-derived CO_2, and plant composition influence the δ^{13}C of surficial layers (Ficken et al., 1998; Pancost et al., 2000), and the resulting δ^{13}C signal is subsequently altered further during diagenesis of these buried peat soils over time~~ transferred to deeper layers.~~. Topography ~~will~~could also influence δ^{13}C because oxygen availability decreases with increasing water depth, resulting in different levels of methanogenesis and methanotrophy in hummocks versus hollows. Topography further influences δ^{13}C because hummock C is older than hollow C for equivalent depths below the mean bog surface. The anthropogenic addition of ^{13}C-depleted CO_2 to the atmosphere via the burning of fossil fuels (the Suess effect, Ehleringer et al., 2000) also increases the gradient between ^{13}C-depleted surficial horizons and older, ^{13}C-enriched deeper horizons.

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How N dynamics will influence δ^{15}N patterns is also shown in Figure 1. In aerobic soils, uptake by mycorrhizal fungi and subsequent transfer of ^{15}N-depleted N to host plants increases the ^{15}N divergence between deeper, ^{15}N-enriched horizons and surficial horizons (Hobbie and Ouimette, 2009), with such processes presumably not operating in *Sphagnum* and deep-rooted nonmycorrhizal plants (Kohzu et al., 2003~~).~~), but potentially operating in forested bogs. Nitrogen transport from uplands can be considerable in the lagg drainage region surrounding a peatland (Verry and Janssens, 2011), and depending on the drainage δ^{15}N, may influence the δ^{15}N of the receiving peatland. For example, lagg drainage could contribute ^{15}N-depleted nitrate or ^{15}N-enriched dissolved organic N (DON) (~~Yano et al., 2009~~Kalbitz and Geyer, 2002), that differ isotopically from N fixation (0‰) or atmospheric N inputs (Stewart et al., 1995; Högberg, 1997). In addition, biogeochemical hotspots are important for N dynamics in peatlands (Hill et al., 2016). Microbial processing of organic matter in soils commonly increases the δ^{15}N and δ^{13}C of the residual material (Nadelhoffer and Fry, 1994), although a N loss mechanism must also be present for δ^{15}N to be affected. Such processing decreases the C/N of organic matter, since respiratory C losses are generally greater than N losses.

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Here, we used δ^{15}N, δ^{13}C, %N, and %C patterns of peat profiles, plant tissues, and fungal hyphae sampled from the Spruce and Peatland Responses Under Climatic and Environmental Change (SPRUCE) experimental site in northern Minnesota, USA at the Marcell S1 bog ~~(prior to initiation of a global change experiment, Iversen et al., 2014)~~ to investigate potential factors influencing C and N turnover in peatlands. In addition to the continuous variables of elemental concentration ~~and~~, isotopic signatures, and depth, nominal variables included plot location~~, depth~~, topography (hummock versus hollow), and vegetation (near trees or not). We used concurrent radiocarbon measurements (~~Mcfarlane~~Iversen et al., ~~*submitted*~~2014) to link the stable isotope measurements to the 11,000-year history of C and N dynamics at the SPRUCE experimental site. With this combination of data, we studied how *in situ* biogeochemistry and peatland succession may have influenced the isotopic profiles. We inferred the path of peatland succession from a prior study of the nearby S2 bog, as given in Table 1. Successional history at S1 should be similar, with the vegetation proceeding from a rich fen to a transitional fen ~5500 years BP, transitioning to a poor fen ~2900 years BP, and then changing to a forested bog ~400 years BP.

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Isotopic patterns reflect ~~the sum total of~~ numerous ~~factors. Specifically~~biogeochemical processes. Here, we ~~address five~~addressed four potential drivers of isotopic variation in a boreal peatland profile:

(1) in upper peat layers, ^{13}C depletion will reflect anthropogenic declines in the δ^{13}C of atmospheric CO_2 (Suess effect);

(2) microbial processing and biochemical composition (as inferred from %N, %C, and C/N) will influence peatland δ^{13}C and δ^{15}N;

(3) proximity to uplands will increase N concentrations and peat δ^{15}N;

(4) ~~climatic patterns during the Holocene may influence~~ peatland ~~δ^{13}C;~~

~~(5) methanogenesis in~~colonization by ectomycorrhizal trees will increase peat ~~profiles during periods of high sedge abundance will enrich ^{13}C in~~δ^{15}N and the ^{15}N enrichment between surficial and deeper peat ~~whereas during periods of low sedge abundance methanotrophy combined with subsequent CO_2 assimilation by *Sphagnum* will deplete ^{13}C in peat.~~

In addition to these drivers, we will examine the covariation of peat δ^{13}C and δ^{15}N with climate or vegetation through the Holocene.

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2 Methods

2.1 Site description

Soil and fungal samples were collected from the SPRUCE experimental site at the 8-hectare S1 bog in the USDA Forest Service Marcell Experimental Forest in northern Minnesota, USA (47° 30.476’N, 93° 27.162’W). The bog is dominated by the trees *Picea mariana* (Mill.) Britton, Sterns and Poggenb. and *Larix laricina* (Du Roi) K.Koch, ericaceous shrubs (*Ledum groenlandicum* Oeder; *Chamaedaphne calyculata* (L.) Moench.) and *Sphagnum* mosses. Various forbs and sedges are also present. The bog topography can be separated into hummocks (protruding above the average water table) and depressed hollows, and divided into areas with

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trees (*Picea* or *Larix*) or without trees. Average annual air temperature from 1961 to 2005 was 3.3°C with yearly mean extremes of -38°C and 30°C and average annual precipitation of 768 mm (Sebestyen et al., 2011). Average pH of the peat is 4.1 and average gravimetric water content is 7.40 g H_2O g^{-1} dry peat (Iversen et al., 2014). The water table fluctuates about 30 cm annually and water table depth can reach 1.4 m over the course of a 50-year record (Sebestyen et al., 2011). The bog maintains a perched water table with a hydraulic gradient about 1 m above the surrounding aquifer, which is composed of outwash sand (Verry et al., 2011).

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2.2 Procedures

Peat cores for this analysis were collected in mid-August of 2012 from locations along three boardwalks extending out into the bog beyond the lagg region (Figure 2). Surface peat (~0–30 cm) was collected using a modified hole saw, while deeper ~~peat~~samples down to mineral soil (~30–250 cm) ~~was~~were collected using a Russian peat corer. Cores were taken in both hummocks and hollows, with 0 cm defined as the surface of hollows and hummock heights above that assigned positive depths. Cores were bulked and homogenized every 10 cm over the 0 to -100 cm depth, every 25 cm from -100 to -200 cm, and over the entire 50 cm increment from -200 to -250 cm (in some cases, -300 cm was reached before mineral soil was observed). Cores were sampled at 17 locations (Figure 2; the locations of experimental plots distributed across the three boardwalks) and material from 16 of these locations was used for the ~~δ~~δ^{13}C, ~~δ~~δ^{15}N, and radiocarbon measurements reported here. At locations 4, 5, 6, and 7 along the southern boardwalk, separate cores were taken within 150 cm of *Picea* or *Larix* trees and in the open (no trees within 150 cm), and the distinction designated as ‘treed’ or ‘untreed’.

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Peat cores were analyzed for Δ^{14}C, δ^{13}C, δ^{15}N, %C, %N, and C/N by depth increment, with the depth increment recorded as the average depth (for example, 0 to 10 cm in a hummock given as 5 cm). Peat cores were analyzed in hummocks to a depth of -10 cm and in hollows to the bottom of the core (between -200 and -300 cm). Live woody plant foliage and fine roots to -10 cm were collected in August 2012 and live *Sphagnum* in 2013.

To collect fungal hyphae, in-growth cores were constructed. Mesh (40 µm) in-growth bags (10 cm × 10 cm) were filled with sterile sand. Bags were incubated in the field in paired hummock and hollows at six locations in the bog. In hummocks, bags were inserted at +10 to 0 cm above the adjacent hollow and in both locations from 0 to -10 cm and -10 to -20 cm below the hollow surface. Bags were installed on June 5, 2013 and recovered on September 20, 2013. Sand from in-growth bags was combined with ultrapure water and mixed at 80 rpm for 20 minutes. Suspended hyphae were removed with tweezers and the process was repeated until all hyphae were collected. Hyphal biomass was dried in the oven at 60ºC for 48 hours. Of 30 in-growth samples, 20 generated enough hyphal mass for analysis. All 20 samples were treated as independent replicates in statistical analyses.

2.3 Isotopic and elemental analysis

Radiocarbon content of homogenized bulk peat was measured on the Van de Graaff FN accelerator mass spectrometer (AMS) at the Center for AMS at Lawrence Livermore National Laboratory. Peat samples were not chemically pretreated prior to ^{14}C measurement. Samples were prepared by sealed-tube combustion to CO_2 in the presence of CuO and Ag and then reduced onto Fe powder in the presence of H_2 (Vogel et al., 1984). Radiocarbon isotopic values had an average AMS precision of 2.6‰ and were corrected for mass-dependent fractionation with $\delta^{13}C$ values from analyses conducted at the Department of Geological Sciences Stable Isotope Laboratory at University of California-Davis using a GVI Optima Stable Isotope Ratio Mass Spectrometer. Radiocarbon values are reported here in $\Delta^{14}C$ notation corrected for ^{14}C decay since 1950 (Stuiver and Polach, 1977). Calibrated ages were determined using Calib (http://calib.qub.ac.uk/calib/) or CaliBomb (Reimer et al., 2004) with INTCAL13 (Reimer et al., 2013) and Northern Hemisphere Zone 1 bomb curve extension (Hua et al., 2013) atmospheric ^{14}C calibration curves. Years before present (BP) refer to years prior to 1950. For more recent samples, calendar years AD may also be used.

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These same soil samples and additional samples of hyphae and foliage were analyzed for %C, %N, $\delta^{13}C$, and $\delta^{15}N$ at the University of New Hampshire Stable Isotope Laboratory using a Costech 4010 Elemental Analyzer coupled to a Thermo Delta Plus XP IRMS. Standard deviations of laboratory standards (tuna, NIST 1515, and NIST 1575a) for $\delta^{15}N$ and $\delta^{13}C$ averaged less than 0.2‰. Fine roots of the woody vascular plants were analyzed for their stable isotopic composition at the Oak Ridge National Laboratory on an Integra CN mass spectrometer (SerCon, Crewe, UK), using standards traceable to NIST 8547-ammonium sulfate or 8542-sucrose (NIST, Gaithersburg, Maryland, USA).

2.4 Statistical tests

The statistical program JMP (SAS Institute, Middleton, Massachusetts, USA) was used for statistical analyses. Reported values are ± standard error, unless otherwise specified. Regression models for soil $\delta^{13}C$ and $\delta^{15}N$ were tested. Factors included in the regression model included nominal variables of vegetation type (treed or non-treed~~) and~~), topography (hollow or hummock~~). Plot~~), and plot number~~ and depth were also treated as nominal variables. As a nominal variable, depths~~. Depths with only a single measurement were generally excluded, unless they were very similar in depth to another value. Continuous variables included %N, %C, and isotopic values.

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To test whether plot location, proximity to trees, depth, topography, and elemental concentrations influenced the carbon and N isotope patterns in peat profiles, we used multiple regression analyses. Sample %C, %N, C/N, and either $\delta^{13}C$ or $\delta^{15}N$ were included as continuous variables. Because the effects of depth or plot location on N and C dynamics are unlikely to change continuously (for example, methanogenesis requires an anaerobic soil and plots at bog edges may have different hydrology and N dynamics than plots in the middle of the bog, Urban and Eisenreich, 1988), ~~depth and plot were treated as nominal (categorical) variables in our regression~~

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~~models.~~plot was treated as a nominal (categorical) variable in our regression models. To avoid over-parameterizing the model, depth was treated as a continuous variable with a cubic transformation (that is, the regression model included model depth, (model depth)^2, and (model depth)^3 as additional parameters). Stepwise regression was used and model selection based on the lowest Aikike Information Criteria with a correction for sample size (AICc). Within a given depth, values for radiocarbon were tested for correlations against δ^{13}C and δ^{15}N and the slope of the regression estimated.

3 Results

3.1 δ^{13}C and δ^{15}N in plants and fungal hyphae

Of the six vascular plant taxa tested, δ^{13}C of foliage varied from -30‰ in *Larix* to -28‰ in *Picea*. The δ^{15}N of plant foliage varied more widely than δ^{13}C, from -8.5‰ for *Picea* to 2.5‰ for *Eriophorum*. Fine root δ^{13}C averaged -27.4±0.3‰ for *Larix*, -26.9±0.1‰ for *Picea*, and -28.5±0.2‰ for shrubs. Fine root δ^{15}N averaged -4.7±0.4‰ for *Larix*, -4.1±0.3‰ for *Picea*, and -1.8±0.1‰ for shrubs, whereas coarse roots of shrubs averaged -3.4±0.3‰. If we assume plant productivity patterns are similar aboveground and belowground, then the productivity-weighted average in vascular plants for δ^{13}C was -29.2‰ for foliage and -27.3‰ for roots (Table 2). Fungal hyphae from in-growth cores (n = 20) averaged -26.0±0.2‰ (se) for δ^{13}C and -0.3±0.2‰ for δ^{15}N.

3.2 δ^{13}C and δ^{15}N in peat profiles

Carbon isotope (δ^{13}C) values of peat in the profile increased from -29‰ in the top 10 cm of hummocks and hollows to -26‰ at -112 cm and then decreased slightly at greater depths. δ^{13}C values changed most rapidly from 0 cm to -50 cm depth (Figure 3a). Nitrogen isotope values in the peat profile increased from -3‰ in hummocks above the water level to around 1‰ at -50 cm. δ^{15}N then decreased to 0‰ at -85 cm before increasing again to 1.5‰ at -200 cm. Similar to δ^{13}C, δ^{15}N changed most rapidly from 0 cm to -50 cm depth (Figure 3b).

In a stepwise regression model for δ^{13}C including δ^{15}N, C/N, %N, %C, and ~~%C~~depth as continuous variables and the vegetation type, topography, and plot sampling location~~, and depth~~ as nominal variables, all factors were ~~statistically significant.~~retained in our final model, including four terms for partitioning the 16 plots. The model explained ~~86~~85% of the total variance (n = 238, adjusted r^2) in peat δ^{13}C. ~~Depth~~The three depth terms (depth, depth squared, and depth cubed) explained ~~49~~45%, %N explained ~~12~~10%, C/N ~~and~~11%, δ^{15}N ~~each~~15%, %C explained ~~11%,~~2%, the four plot terms explained ~~10~~7%, and ~~the remaining three variables together~~ proximity to trees explained ~~just 7~~3% of the variance (Table 3). ~~In the regression model, plot 17 was significantly higher and plots 4, 5, and 19 were significantly lower than the mean for δ^{13}C. The complete regression models for~~Topography explained

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10% of variance, with hollows lower than hummocks by 0.89‰ in δ^{13}C ($p < 0.001$). Results of the stepwise model testing δ^{13}C and δ^{15}N are given in ~~Appendix 1.~~ Table A1.

~~A~~The stepwise regression model for δ^{15}N of peat included δ^{13}C~~, C/N, %N,~~ and ~~%C~~the same variables as ~~continuous variables and the vegetation type, topography, plot, and depth as nominal variables.~~in the δ^{13}C regression model. This model explained ~~70~~66% of the total variance (adjusted r^2~~).~~), with proximity to trees and topography dropping out of the regression model. Of the explained variance, ~~depth accounted for 39%, %N 15%, δ^{13}C and plot 14% each, C/N 10%, and the remaining three variables accounted for 9% (Table 3). For δ^{15}N, plots 4, 9, and 19 were significantly above 0‰ and plot 11 was significantly below 0‰. Hollows were lower than hummocks by 0.64‰ in δ^{13}C (p = 0.007) and by 1.42‰ in δ^{15}N (p = 0.008), and cores near trees were lower by 0.30‰ in δ^{13}C (p = 0.014) and higher by 0.58‰ in δ^{15}N (p = 0.029) than those without trees.~~the three depth terms accounted for 8%, %N 22%, %C 7%, C/N 17%, δ^{13}C 34%, and the three plot terms 11%, (Table 3).

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The ~~coefficients for the nominal variable~~influence of depth and ~~plot~~location in our regression models ~~explaining~~on δ^{13}C and δ^{15}N values ~~are~~is plotted in Figure 4 and Figure 5, respectively. ~~Coefficients for~~The influence of depth ~~were~~was negative above 0 cm (corresponding to the surface of the hollows), increased regularly from -5 cm to -25 cm, and then varied little in δ^{13}C in the deepest horizons while still increasing in δ^{15}N. ~~For δ^{13}C, most depth coefficients differed significantly from 0 except at 5, -15, and below -162 cm, whereas for δ^{15}N, all depth coefficients differed significantly from 0 except at -25 cm, -65, -85 cm, and below -225 cm. The depth coefficients for δ^{13}C and δ^{15}N were positively correlated (adjusted $r^2 = 0.306$, $n = 17$, $P = 0.0125$, $\delta^{15}N = 1.64 \pm 0.58 \times \delta^{13}C + 0.00 \pm 0.43$) (Figure 4). The coefficients for the nominal variable of plot in models explaining δ^{13}C and δ^{15}N values were negatively correlated (adjusted $r^2 = 0.279$, $p = 0.02$, $\delta^{15}N = -1.34 \pm 0.51 \times \delta^{13}C + 0.07 \pm 0.08$, Figure 5a). There was some spatial patterning of values, with plots near to the western upland high in δ^{15}N and low in δ^{13}C, and other plots showing the opposite pattern (Figure 5b~~There was some spatial patterning of values across plots, with two plots (4 and 19) near to the western upland high in δ^{15}N (Figure 2 and Figure 5).

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Although overall patterns of radiocarbon with depth were clear, radiocarbon varied widely at any given depth, and correlated significantly with δ^{13}C or δ^{15}N at several depths (Table 4). Radiocarbon correlated positively with δ^{13}C at -~~163~~162 cm, -5 cm, 5 cm, and 15 cm, and correlated negatively with δ^{13}C at -35 cm and -65 cm. In contrast, radiocarbon correlated positively with δ^{15}N at -65 cm, -55 cm, -45 cm, - 5 cm, and 15 cm, and correlated negatively with δ^{15}N at -162 cm and -25 cm. Overall patterns of Δ^{14}C with δ^{13}C or δ^{15}N are shown in Figure 6a and Figure 6b, respectively.

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4 Discussion

4.1 Potential causes of shifts in δ^{13}C and δ^{15}N in peat profiles

Isotopic ratios within the profile can shift if elemental fluxes in or out of the system differ isotopically from profile material (Figure 1). Loss of labile C via respiration, methanogenesis, or leaching (Kolka et al., 1999) could alter the δ^{13}C of the residual material, as could inputs of ^{13}C-enriched material such as roots or mycorrhizal hyphae. Similarly, changes in the δ^{13}C of atmospheric CO_2 can alter the δ^{13}C of photosynthetically fixed C whereas changes in moisture, temperature, or photosynthetic capacity can alter the ^{13}C discrimination between atmospheric CO_2 and fixed C. For N, loss of ^{15}N-depleted material from the bulk peat via mycorrhizal transfer to fine roots, direct root uptake, denitrification, or leaching of organic or inorganic N could raise the δ^{15}N of the remaining soil organic matter. Inputs of N via atmospheric deposition, N fixation, or transport from surrounding uplands could also influence δ^{15}N if these inputs differ isotopically from peat profile values. These processes can be linked to past climate and vegetation with profile radiocarbon measurements that are calibrated to calendar years. Here, we used radiocarbon to indicate the potential timing of shifts in some of the primary drivers that influenced C and N stable isotope patterns within the peat profiles at SPRUCE~~. We will first discuss quantitative factors within~~, such as the ~~framework of our multiple regressions of δ^{13}C~~Suess effect and ~~δ^{15}N, then discuss~~ the ~~categorical variables of vegetation cover, topography, plot,~~transition to a forested bog, but also examined processes that were not tied to a particular time period, such as differences in C and ~~depth.~~ N stoichiometry or proximity to uplands.

4.2 %N, %C, and C:N stoichiometry ~~influence~~influenced δ^{13}C and δ^{15}N patterns

%N, %C, and C/N contributed ~~26~~a combined 47% and ~~29~~23% of explained variance, respectively, to our regression models of δ^{15}N and δ^{13}C. These factors ~~reflect~~reflected the biochemical and isotopic composition of the original plant material, ~~or may reflect~~but also reflected how the chemical structure and isotopic composition of plant material has altered during its slow decomposition at S1.

Microbially-driven C loss raises soil organic matter %N, lowers C/N, and enriches soil organic matter in ^{13}C (resulting from loss of ^{13}C-depleted CO_2) (Ehleringer et al., 2000; Alewell et al., 2011). The positive correlation of %N with δ^{13}C may therefore reflect an underlying correlation between the accumulation of ^{13}C-enriched microbial necromass (Wallander et al., 2004) and the increased N content of the peatland organic matter. Fungal %N and δ^{13}C are positively correlated (Hobbie et al., 2012) because of the high δ^{13}C of microbially synthesized protein relative to other microbial components such as carbohydrates and lipids. In contrast, the positive correlation of C/N with δ^{13}C and negative correlation with δ^{15}N presumably reflects a legacy of buried wood, which, relative to other plant material, should be high in δ^{13}C (Trudell et al., 2004) and high in C/N. This can be seen clearly in the few samples with C/N greater than 70, which is higher than any plant tissue measured in this study. Although some *Sphagnum* taxa under pristine conditions can be very low in %N (0.22%) and δ^{15}N (-3.6‰) and high in C/N (~190) (Asada et al., 2005a), here, the presence of wood was noted seven times during laboratory examination of the 238 samples at depths from 15 cm to -35 cm, with those samples twice as high in C/N (average, 69) as other samples, and were also significantly higher in C/N in multiple regression analysis (see ~~Appendix 2~~Table A2).

The negative correlation of %N with δ^{15}N indicated that either added or removed N is low in δ^{15}N. One possibility for removal is that ^{15}N-depleted N has been transferred from mycorrhizal fungi to plants~~ and subsequently lost during fire or as DON after decomposition at the surface. By removing the surficial, ^{15}N-depleted litter horizons, fire enriches the soil profile in ^{15}N (Hobbie and Högberg,~~

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~~2012).~~. Alternatively, N could be added via fixation with a $\delta^{15}N$ value of -1‰, which would lower overall $\delta^{15}N$ values deeper in the peat profile. However, we point out that only at -45 cm and -55 cm are %N and $\delta^{15}N$ significantly and negatively correlated (~~Appendix 3~~Table A3). At these depths, %N is about 1.8% and $\delta^{15}N$ is about 1‰ (Figures 3b, d). The value of the coefficient for %N in the $\delta^{15}N$ regression, -2.~~6‰/%~~96‰ %N~~$^{-1}$~~, implied that the perturbing N has a $\delta^{15}N$ value that is 1.8% × 2.~~6‰/%~~96‰ %$^{-1}$ less than that of 1‰, or -4.3~~.68~~‰, which is too low to be fixed N. The apparent ^{15}N depletion of ~~4.7~~5.3‰ against the source N is a plausible value for ^{15}N discrimination between mycorrhizal fungi and host plants (Hobbie and Colpaert, 2003). ~~In addition, DON appears to be higher in $\delta^{15}N$ than bulk peat (Broder et al., 2012), so DON losses could not cause a ^{15}N enrichment of the remaining N. It also implies that N fixation must be above this depth, which agrees with the apparent coupling of N fixation and *Sphagnum* activity (Bragina et al., 2012; Leppänen et al., 2015).~~

Correlations of carbon concentration with stable isotopes provide information about chemical composition or degree of processing. The negative correlation of %C with $\delta^{13}C$ is expected based on the chemical composition of ^{13}C-depleted compound classes of lignin, aromatics, and lipids, which are high in %C (Poorter et al., 1997; Hobbie et al., 2002). Initial decomposition of *Sphagnum* commonly decreases $\delta^{13}C$, as ^{13}C-enriched soluble components are leached (Asada et al., 2005b). In contrast, aromatics and lipids do not generally contain N, so the positive correlation of %C with $\delta^{15}N$ in bulk peat cannot be explained in the same manner. However, microbial processing generally enriches soils in ^{15}N (Billings and Richter 2006; Templer et al., 2007) while increasing %C, which was also true at S1 (Tfaily et al., 2014).

4.3 ~~$\delta^{13}C$ and $\delta^{15}N$ patterns are linked~~

~~Information about the structure of the compounds isotopically fractionated during peat decomposition can be inferred from the relative effects of $\delta^{15}N$ and $\delta^{13}C$ in our regression models on $\delta^{13}C$ and $\delta^{15}N$. The positive effect of $\delta^{15}N$ on $\delta^{13}C$ values (slope, 0.177) and $\delta^{13}C$ on $\delta^{15}N$ values (slope, 0.882) is not surprising, since C:N bonds are ubiquitous in organic material and breaking a C:N bond will discriminate against both ^{13}C and ^{15}N (Silfer et al., 1992). The coefficient of $\delta^{15}N$ in the multiple regression for $\delta^{13}C$ can be defined as $\delta^{15}N_{coeff}$ and can be expressed as:~~

~~$\delta^{15}N_{coeff} = \varepsilon^{13C}/\varepsilon^{15N} \times f_{C:N}$ Eq. (1)~~

~~where ε^{13C} and ε^{15N} are the ^{13}C and ^{15}N enrichments during C:N bond formation or breaking, and $f_{C:N}$ is the proportion of total C bonds fractionated that involve a C:N bond.~~

~~Similarly, we can write the coefficient for the effect of $\delta^{13}C$ on $\delta^{15}N$ as:~~

~~$\delta^{13}C_{coeff} = \varepsilon^{15N}/\varepsilon^{13C} \times f_{N:C}$ Eq. (2)~~

~~where $f_{N:C}$ is the proportion of total N bonds fractionated that involve a C:N bond. Combining these two equations results in:~~

~~$\delta^{15}N_{coeff} \times \delta^{13}C_{coeff} = f_{C:N} \times f_{N:C}$ Eq. (3)~~

~~Replacing $\delta^{15}N_{coeff}$ with 0.177 and $\delta^{13}C_{coeff}$ with 0.882 gives a value of $f_{C:N} \times f_{N:C}$ of 0.156, so on average 40% of bond fractionations ($0.156^{0.5}$) involve C:N bonds. Interestingly, comparable data were generated from $\delta^{15}N$ and $\delta^{13}C$ patterns in caps and stipes of mushrooms (Hobbie et al., 2012). In those multiple regressions, $\delta^{15}N_{coeff}$ was~~

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~~0.121 and $\delta^{13}C_{coeff}$ was 0.853, so $f_{C:N} \times f_{N:C}$ would be 0.103 and on average 32% of bond fractionations ($0.103^{0.5}$) involved C:N bonds. The somewhat higher proportion of bond fractionations involving C:N bonds at S1 (40%) than in pure fungi (32%) may simply reflect the greater contributions to isotopic patterns of microbial biomass from Archaea and Bacteria at S1, since these latter taxa have higher N:C ratios and protein content than fungi, and therefore more peptide bonds. Since peptide bonds link C and N, peptide bond disruption will affect C and N bonds equally.~~

~~4.4 The Suess effect increases ^{13}C depletion in surficial peat~~

~~The strong dependence of $\delta^{13}C$ on peat depth partially reflects the 1.7‰ decline in the $\delta^{13}C$ of atmospheric CO_2 since 1850, with the lowest $\delta^{13}C$ values above the water table, where C is of recent origin. For example, the lowest values of depth coefficients (~-1‰) in hummocks at 15 cm, 22 cm, and 25 cm above the mean bog surface reflect C from the last 50 years, as confirmed by $\Delta^{14}C$ averages of 59‰, 29‰, and 52‰ for these three depths, where only C influenced by ^{14}C created during thermonuclear testing should have positive $\Delta^{14}C$ values (Table 4). Although our sampling lacked sufficient vertical resolution to explicitly include corrections for the Suess effect (e.g., as done in Esmeijer-Liu et al., 2012), the ~1.5‰ increase in the depth coefficient of our $\delta^{13}C$ regression model from the hummocks to deeper in the profile correspond well to the long-term shift in $\delta^{13}C$ of atmospheric CO_2 from pre-industrial times to the present. An additional factor contributing to the higher depth coefficient could be the 1-2‰ higher $\delta^{13}C$ in roots than in foliage and the different input depths of foliage (surface only) and roots (distributed throughout the acrotelm). The steady increase in the $\delta^{13}C$ coefficient between -5 cm and -25 cm depth presumably reflects the increasing dominance of pre-industrial C. Depths of -35 cm and below all had $\Delta^{14}C$ values less than -100‰ (Table 4), indicating primarily pre-bomb and pre-industrial C when the average $\delta^{13}C$ of atmospheric CO_2 was -6.5‰ (versus the current value of -8.2‰). In addition, modern production of organic matter averaged -29‰ in $\delta^{13}C$ (Table 2), similar to values for surficial horizons, whereas deeper horizons were between -27‰ and -26‰. The Suess effect of ~1.5‰ therefore accounted for at least half of this difference.~~

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~~4.5~~ Proximity to uplands and trees ~~increases~~increased peat $\delta^{15}N$

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Plot-specific coefficients for $\delta^{13}C$ and $\delta^{15}N$ ~~are negatively correlated (Figure 5a), and may therefore~~may reflect site-specific differences in the dominance of conditions favoring ^{13}C- or ^{15}N-depleted losses during peatland development. The positive coefficients for $\delta^{15}N$ ~~and negative coefficients for $\delta^{13}C$~~ are from ~~the~~two plots, 4 and 19, closest to the lagg region adjacent to the western upland. This suggests that the different hydrology in the lagg has enhanced ^{15}N fractionation from N removal mechanisms such as denitrification, nitrification, or leaching of DON. Alternatively, dissolved N transported from the uplands during spring thaw and melt may have provided an additional ^{15}N-enriched N source for ~~these~~ plots 4 and 19 located near the bog edges (Figure ~~5b~~5). Peatland DON appears enriched in ^{15}N and ^{13}C relative to bulk peat (Broder et al., 2012), and this is presumably true for upland sources as well. In the adjacent Marcell S2 kettle bog, large N fluxes from upland locations from both surface runoff and interflow led to much larger N losses in streamflow from the lagg region (~32 kg ha^{-1} yr^{-1}) than from the bog itself (2 kg ha^{-1} yr^{-1}) (Urban and Eisenreich, 1988). The uplands here are dominated by ectomycorrhizal trees such as

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Populus, *Quercus*, and *Pinus*, which ~~tend to~~ produce vertically stratified soil profiles with high δ^{15}N values in lower organic and mineral horizons (Hobbie and Ouimette, 2009). We therefore expect DON produced in uplands to be high in δ^{15}N, which will serve as a source of ^{15}N-enriched N to lagg regions of peatlands. ~~The greater vertical stratification of δ^{15}N possible with trees than with mosses may therefore also cause here the higher δ^{15}N coefficient for treed versus non-treed locations (Table 3), since higher levels of ^{15}N-depleted N in the active surficial layers should promote losses of this N and the ultimate ^{15}N enrichment of remaining N. In contrast, the lower δ^{13}C coefficient for treed versus non-treed locations suggests decreased surface wetness near trees and consequent greater ^{13}C discrimination by *Sphagnum* during photosynthesis (Kühl et al., 2010). Relative to *Sphagnum*, the high transpiration rates of trees relative to *Sphagnum* may reduce surface moisture.~~

4.~~6 Isotopic~~4 δ^{15}N patterns with depth ~~reflect~~reflected climate and vegetation

Peatland succession and climate have been established previously at the nearby S2 bog and are summarized in Table 1. As the same climatological factors affected the S2 and S1 (SPRUCE) bogs, plant stratigraphy and isotopic patterns were probably similar, although accumulation rates are lower at S1 than at S2 (~~personal communication, E. Verry). Vegetation cover plays a role in δ^{13}C patterns, as sedges may cause δ^{13}C in peatlands to increase by promoting loss of ^{13}C-depleted methane, thereby increasing δ^{13}C of residual C, whereas mosses and associated methanotrophic bacteria may foster the retention of ^{13}C-depleted C in peatlands dominated by *Sphagnum* mosses (Larmola et al., 2010).~~McFarlane, unpublished data). ~~The high δ^{15}N and relatively low δ^{13}C at -213 cm and -225 cm corresponded approximately to a warm period between 8000 and 9200 BP, during a sedge-dominated rich fen stage (Verry and Janssens, 2011), with mean annual temperatures of 4-5°C. Higher temperatures in peatlands are associated with lower δ^{13}C values (Skryzpek et al., 2005, 2008).~~

In the following paragraphs, we ~~will~~ link shifts in δ^{15}N~~ and δ^{13}C~~ through the profile to radiocarbon ages and the corresponding patterns in vegetation and climate at the S2 bog. The high δ^{15}N at -213 cm and -225 cm corresponded approximately to a warm period between 8000 and 9200 BP, during a sedge-rich fen stage (Verry and Janssens, 2011), with mean annual temperatures of 4-5°C. Given similar C:N ratios during this period to subsequent periods (~20), nitrogen losses were probably more depleted in ^{15}N than subsequent losses, that is, more losses via nitrate leaching or denitrification rather than via DON leaching. Nitrification~~Radiocarbon and δ^{13}C correlated positively at -162 cm, corresponding to a 1‰ rise in δ^{13}C which accompanied a drop in MAT to perhaps 2°C by 6000 BP. This cooling trend was also accompanied by a slight rise in precipitation, so the decreased ^{13}C discrimination could also be attributed to increased *Sphagnum* moisture (Rice and Giles, 1996), although it is difficult to distinguish between these two possible causes of ^{13}C differences (Ménot and Burns, 2001). *Sphagnum* discrimination is less with increased moisture because CO$_2$ diffusion is limited under wet conditions. The stratigraphy at the S2 bog indicates a vegetational shift from a rich fen to a transitional fen during this period. The negative correlation between radiocarbon and δ^{15}N at this depth suggests decreasing losses of ^{15}N-depleted N during this vegetational transition.~~

~~Based on plant stratigraphy at the neighboring S2 bog, the depth increment from -85 cm to -112 cm corresponds to a transitional fen stage 3300-4800 years ago. In our regression model for δ^{13}C, these two depths are about 0.5‰ higher in δ^{13}C than at -162 cm. The peak in δ^{13}C may reflect a phase during which sedges transported~~

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~~methane directly to the atmosphere, thereby minimizing the refixation in *Spaghnum* cells of ^{13}C-depleted, methanotrophic-derived carbon dioxide. An accompanying trough in δ^{15}N at -400‰ Δ^{14}C (4220 calibrated years BP, Figure 5) suggests that processes increasing δ^{13}C such as high sedge abundance may decrease sequestration of ^{15}N-enriched organic matter. One possible explanation is that systems with nonmycorrhizal sedges create less ^{15}N-depleted N in the active surficial horizons because of sedge uptake of deeper, ^{15}N-enriched N, as suggested by high *Eriophorum* δ^{15}N relative to *Sphagnum* and other vascular plants (Table 2) and the deep root distribution of sedges relative to woody plants (Iversen et al., 2015). As a consequence, N losses via equivalent pathways (e.g., denitrification, DON) from sedge systems will tend to be ^{15}N-enriched relative to N losses from systems without sedges.~~

~~The positive correlations between δ^{15}N and Δ^{14}C at -45, -55, and -65 cm (Table 4 and Figure 6b) may be linked to a parallel decline in %N over these time periods, so that younger samples are lower in %N and higher in δ^{15}N than older samples. This presumably reflects losses of ^{15}N-depleted N from the younger samples.~~

~~The cause for the drop of 1.4‰ in the δ^{15}N coefficient from -35 cm depth (modern) to -25 cm (~1000 yr BP) and greater depths is unclear; possibly, since there is no parallel increase in the δ^{13}C coefficient, this reflects long-term cycling and loss of N during denitrification, as suggested by Puglisi et al. (2014). However, nitrate is very low at this site (Iversen et al., submitted) and is very low in organic horizons in bogs in other studies (Bayley et al., 2005). Nonetheless, nitrification~~ and denitrification are higher in fens than in bogs and should change δ^{15}N patterns along the core profile as the core reflects peatland succession and climatological changes (Regina et al., 1996; Bayley et al., 2005; Wray and Bayley, 2007).

The S2 bog shifted from a rich fen to a transitional fen by 5600 years BP, while MAT decreased to perhaps 2°C. At the S1 bog, the negative correlation between radiocarbon and δ^{15}N at -162 cm (~6000 years BP) suggested decreasing losses of ^{15}N-depleted N during this transition. The depth increment from -85 cm to -112 cm corresponded to a transitional fen stage 3300-4800 years BP. The accompanying trough in δ^{15}N at -400‰ Δ^{14}C (4220 calibrated years BP, Figure 6b) suggested decreased sequestration of ^{15}N-enriched organic matter as nutrient availability declined during this transition.

The positive correlations between δ^{15}N and Δ^{14}C at -45, -55, and -65 cm (Table 4 and Figure 6b) are linked to a parallel decline in %N over these time periods, so that younger samples are lower in %N and higher in δ^{15}N than older samples, reflecting losses of ^{15}N-depleted N from the younger samples. δ^{15}N values peaked at -35 to -55 cm, and then declined above and below those depths. This pattern suggested that those depths were affected by a ^{15}N enrichment process that did not affect deeper depths (that is, further in the past). The peak δ^{15}N accordingly reflected ^{15}N partitioning between surficial and deeper horizons by a new mechanism.

We suggest that ectomycorrhizal fungi are the most probable cause of this unusual peak in δ^{15}N at intermediate depths in the peat bog. At the nearby S2 bog, poor fen transitioned to forested bog between 1610 AD and 1864 AD, with a charcoal layer at S2 indicating that peat was consumed by fire during this period, precluding a more specific date for this transition (~~Kolka, 2011; Verry and Janssens, 2011).~~ Verry and Janssens, 2011). This forested bog is dominated by ectomycorrhizal *Picea* and *Larix*. Transfer to plants of ^{15}N-depleted N by ectomycorrhizal fungi leads to low plant δ^{15}N and high ^{15}N enrichment of the residual N not transferred (Hobbie and Hobbie, 2008; Hobbie and Högberg, 2012). Here, the apparent influence of ectomycorrhizal uptake can be traced to ~-55 cm.

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~~Here, negative values of the depth coefficient for δ^{15}N in the hummocks (heights from 5 to 25 cm above the hollows) reflect the low δ^{15}N of recent litter inputs from ectomycorrhizal and ericoid mycorrhizal plants to these layers, as seen in Table 2, and the ~6‰ higher value for fungal hyphae. Transfer to plants of ^{15}N-depleted N by ectomycorrhizal and ericoid mycorrhizal fungi creates this contrast between low plant δ^{15}N and high fungal δ^{15}N (Hobbie and Hobbie 2008; Hobbie and Högberg, 2012). However, the~~The relatively low contribution of ectomycorrhizal and ericoid mycorrhizal fungi to total plant uptake here compared to forest sites dominated by ectomycorrhizal fungi accounted for the lower ^{15}N enrichment between surficial layers and deeper layers here (~3‰) than in forest soils, which averaged a 9.6‰ enrichment between surficial and deeper soils for ectomycorrhizal forests (reviewed in Hobbie and Ouimette, 2009). ~~A relatively small influence of N uptake by ectomycorrhizal~~Ectomycorrhizal and ericoid mycorrhizal trees and shrubs ~~on profile δ^{15}N patterns was observed~~contributed relatively little to total N uptake at the nearby Marcell S2 bog, where mosses contributed 75% of total plant uptake, herbaceous plants 13%, and mycorrhizal trees and shrubs 13% (Urban and Eisenreich, 1988). A few studies in peatlands have reported δ^{15}N values through profiles, but those values have peaked at -1‰ to 0‰ and have been in systems without ectomycorrhizal trees present (Krüger et al., 2015; Esmeijer-Liu et al., 2011).

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4.5 δ^{13}C patterns with depth reflected climatic factors

Once shifts in δ^{13}C caused by compositional shifts are removed, the remaining patterns can be explained by invoking climatic or vegetation shifts. The relatively low δ^{13}C at -213 cm and -225 cm corresponded to a warm period between 8000 and 9200 BP, during a sedge-dominated rich fen stage (Verry and Janssens, 2011), with mean annual temperatures of 4-5°C. Higher temperatures in peatlands are associated with lower δ^{13}C values (Skryzpek et al., 2005, 2008).

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Radiocarbon and δ^{13}C correlated positively at -162 cm, corresponding to a 1‰ rise in δ^{13}C which accompanied a drop in MAT to perhaps 2°C by 6000 BP. This cooling trend was also accompanied by a slight rise in precipitation, so the decreased ^{13}C discrimination could also be attributed to increased *Sphagnum* moisture (Rice and Giles, 1996), although it is difficult to distinguish between these two possible causes of ^{13}C differences (Ménot and Burns, 2001). *Sphagnum* discrimination is less with increased moisture because CO_2 diffusion is limited under wet conditions. The stratigraphy at the S2 bog indicated a vegetational shift from a rich fen to a transitional fen during this period.

The depth increment from -85 cm to -112 cm corresponded to a transitional fen stage 3300-4800 years ago. In our regression model for δ^{13}C (Figure 4), these two depths are about 0.5‰ higher in δ^{13}C than at -162 cm. The peak in δ^{13}C may reflect a phase during which sedges transported methane directly to the atmosphere, thereby minimizing the refixation in *Sphaghnum* cells of ^{13}C-depleted, methanotrophic-derived carbon dioxide (Raghoebarsing et al., 2005).

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4.6 The Suess effect increased ^{13}C depletion in surficial peat

The strong dependence of $\delta^{13}C$ on peat depth partially reflected the 1.7‰ decline in the $\delta^{13}C$ of atmospheric CO_2 since 1850, with the lowest $\delta^{13}C$ values above the water table, where C is of recent origin. For example, the lowest estimated values of the $\delta^{13}C$ depth coefficients (~-1‰) in hummocks at 15 cm, 22 cm, and 25 cm above the mean hollow surface reflect C from the last 50 years (Figure 4), as confirmed by $\Delta^{14}C$ averages of 59‰, 29‰, and 52‰ for these three depths, where only C influenced by ^{14}C created during thermonuclear testing should have positive $\Delta^{14}C$ values (Table 4). Although our sampling lacked sufficient vertical resolution to explicitly include corrections for the Suess effect (e.g., as done in Esmeijer-Liu et al., 2012), the ~2‰ increase in the depth coefficient of our $\delta^{13}C$ regression model from the hummocks to deeper in the profile correspond well to the long-term shift in $\delta^{13}C$ of atmospheric CO_2 from pre-industrial times to the present. An additional factor contributing to the higher depth coefficient could be the 1-2‰ higher $\delta^{13}C$ in roots than in foliage and the different input depths of foliage (surface only) and roots (distributed throughout the acrotelm). The steady increase in $\delta^{13}C$ between -5 cm and -25 cm depth (Figure 4) presumably reflected the increasing dominance of pre-industrial C. Depths of -35 cm and below all had $\Delta^{14}C$ values less than -100‰ (Table 4), indicating primarily pre-bomb and pre-industrial C when the average $\delta^{13}C$ of atmospheric CO_2 was -6.5‰ (versus the current value of -8.2‰). In addition, modern production of organic matter averaged -29‰ in $\delta^{13}C$ (Table 2), similar to values for surficial horizons, whereas deeper horizons were between -27‰ and -26‰. The Suess effect of ~1.5‰ therefore accounted for at least half of this difference.

~~4.7 Methanogenesis and methane oxidation~~

~~Although methanogenesis can under certain conditions influence the $\delta^{13}C$ of bulk profiles, as has been reported from Asian rice paddies (Becker-Heidmann and Scharpenseel, 1986), peatlands may be too rich in C for methanogenesis to shift $\delta^{13}C$ values in bulk peat sufficiently to distinguish them clearly from other C loss mechanisms. Annual methane fluxes at S1 are only 2.5% of the fluxes of carbon dioxide (personal communication, P. J. Hanson), so the expected ^{13}C shifts caused by methanogenesis will not greatly affect bulk $\delta^{13}C$. We conclude that given the multiple processes that can influence peatland $\delta^{13}C$, our bulk profile data do not allow us to determine the relative influence of methanogenesis and methane oxidation on $\delta^{13}C$ patterns. Possibly concurrent measurements of bulk deuterium isotopes could settle the issue, since methanogenesis will discriminate greatly against deuterium whereas aerobic C losses will not.~~

5 Conclusions

~~Numerous factors can influence $\delta^{13}C$ and $\delta^{15}N$ patterns in peatland profiles.~~ Although the multiple potential interactions among climate, vegetation, and soil processes ~~make~~made definitive conclusions difficult, we identified several factors that influenced ~~these isotopic~~$\delta^{13}C$ and $\delta^{15}N$ patterns in peatland profiles, including the Suess effect, C and N stoichiometry, microbial processing, ~~and proximity to uplands and trees. The challenge now~~proximity to uplands, and tree colonization. The potential roles of methanogenesis versus respiration in influencing $\delta^{13}C$ could not be addressed using bulk samples, since ^{13}C shifts were relatively small. Future measurements of deuterium

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isotope ratios (δD) would allow separation of effects of respiration (loss of CO_2) versus methane flux on $\delta^{13}C$ patterns, since methanogenesis discriminates against deuterium. Our conceptual model as given in Figure 1 included the Suess effect (influencing $\delta^{13}C$ patterns) and N transport from uplands (probably influencing $\delta^{15}N$ patterns), but missed several additional factors that appeared to be important in controlling the isotopic patterns we reported. Buried wood appeared to influence both $\delta^{13}C$ and $\delta^{15}N$ directly, and the ectomycorrhizal fungi associated with tree species here created a ^{15}N-enriched horizon that may be specific to forested peatlands. The model did not consider N loss mechanisms and associated ^{15}N effects, nor did it consider the potential for ^{13}C differences between roots and aboveground litter to influence $\delta^{13}C$ profiles. One potential way forward is to put these multiple processes into quantitative models of peatland development that includes ~~vegetational~~vegetation succession and climatic drivers, such as the Holocene Peatland Model (Frolking et al., 2010), and to adapt these models to make isotopic predictions that can be compared against data. Such model-data comparisons should continue to improve our ability to interpret isotopic patterns, as well as reveal areas where our model formulations are currently inadequate.

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6 Data availability

The data presented in this study are available in the appendices and through the Iversen et al. (2014) publication.

7 Supplement link (will be included by Copernicus)

The supplement related to this article is available online at http://dx.doi.org/10.3334/CDIAC/spruce.025

Hofmockel, K.S., Chen, J. and Hobbie, E.A. 2016. SPRUCE S1 Bog Pretreatment Fungal Hyphae Carbon and Nitrogen Concentrations and Stable Isotope Composition from In-growth Cores, 2013-2014. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA. DOI: 10.3334/CDIAC/spruce.025

8 Author contributions

Samples were collected by C. Iversen, P. Hansen, and K. Hofmockel and analyzed by J. Chen, E. Hobbie, C. Iversen, and K. Mcfarlane. E. Hobbie prepared the manuscript with contributions from all authors.

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11 Tables

Table 1. Peatland stages at SPRUCE based on stratigraphy at the S2 watershed in the Marcell National Forest, USA (Verry and Janssens, 2011).

Stage	Years BP	Climate
	9300-7400	4-5°C, 700 mm MAT 700
	9200-6500	Warm and dry
Rich fen-sedge peat	8400-5500	
	7400-6100	4°C ~~→~~→ 2°C, MAP 700-800 mm
	6500-6000	Cooling trend
	6100-3200	Stable climate
Transitional fen	5600-3000	
	3200-2400	Cooled slightly
Open poor fen	2900-390	
	2000-1800	Warmer and drier
Little Ice Age	600-150	Cooled 1°C, MAT 800 mm
Forested bog	384/130-now	

Table 2. Average foliar values for $\delta^{13}C$, $\delta^{15}N$, %C, %N, and C/N (n = 7). [1]Weighted average of $\delta^{13}C$ inputs is -29.2~~‰.,~~‰, based on carbon fixation measurements of different taxa (R.J. Norby, personal communication). ANOVA comparing means in vascular plants used a post-hoc Tukey test, with log C/N tested in place of C/N. [2]Data from Tfaily et al. (2014). [3]Top 10 cm only, primarily *Sphagnum*.

Species	$\delta^{13}C\pm se$	$\delta^{15}N\pm se$	%C±se	%N±se	C/N±se	n	% C flux[1]
Chamaedaphne	-29.5 ± 0.1^{c}	-3.8 ± 0.4^{c}	51.30 ± 0.42^{a}	1.50 ± 0.04^{b}	34.53 ± 0.88^{cd}	12	1.5
Eriophorum	-28.4 ± 0.2^{a}	2.5 ± 0.6^{a}	45.16 ± 0.21^{c}	1.45 ± 0.06^{b}	31.49 ± 1.36^{d}	9	--
Larix	-30.4 ± 0.1^{d}	-6.3 ± 0.4^{d}	48.49 ± 0.36^{bc}	0.77 ± 0.06^{c}	65.10 ± 5.17^{b}	7	37.0
Ledum	-29.2 ± 0.1^{bc}	-5.4 ± 0.2^{d}	51.74 ± 0.23^{a}	1.26 ± 0.03^{b}	41.21 ± 1.07^{c}	13	6.6
Picea	-28.0 ± 0.2^{a}	-8.5 ± 0.2^{e}	49.07 ± 0.20^{b}	0.63 ± 0.02^{c}	79.44 ± 3.24^{a}	11	38.3
Smilacina	-28.7 ± 0.2^{ab}	0.1 ± 0.3^{b}	47.20 ± 0.53^{d}	2.46 ± 0.10^{a}	19.53 ± 0.88^{e}	12	1.5
[2]*Sphagnum*	-29.2	-2.0	--	--	--	--	15.0
Hollow[3]	-28.8±0.1	-2.3±0.2					
Hummock[3]	-28.8±0.1	-2.3±0.5					

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Table 3. ~~Regression~~Stepwise regression model for explaining δ^{13}C and δ^{15}N in peat profiles at SPRUCE. ~~*Treed* vs non-treed,~~ Plot, topography (hummock ~~vs.~~versus hollow ~~topography. Plot~~), and ~~depth~~ nearby presence of trees are treated as nominal variables. Value = Coefficient ± standard error; Var~~.~~ = % variance explained. n = 238. ~~Complete regression model including values for specific~~Depth in cm, depth-squared and depth-cubed terms are also included in the model. Plots are sequentially separated into two groups to maximize the variance explained. Plot groups are Group (1), {19&8&11&20&10&15&5 vs. 9&16&921&6&14&4&7&13&17}; Group (2), {15 vs. 5}; Group (3), {9&16&921&6&14&4&7 vs. 13&17}; Group (4), {14&4 vs. 7}; Group (5), {10&6&11&13&9&15 vs. 17&16&5&8&14&20&7&21&19&4}; Group (6), {13 vs. 9}; Group (7), {17&16&5&8&14&20&7&21 vs. 19&4}. Within a group, the first plots ~~and depths are~~ listed have the given ~~in Appendix 1~~value, the second plots listed (after vs.) have the negative of the given value.

δ^{13}C model, adjusted r^2 = 0.~~853~~846, p <0.001 δ^{15}N model, adjusted r^2 = 0.~~701~~660, p <0.001

Source	Value±se	%Var~~.~~	P	Source	Value±se	%Var~~.~~	P
Intercept	-~~27.80~~28.39±0.~~38~~ ~~--~~ ~~--~~37	--	**<0.001**	Intercept	~~25.21±4.09~~ ~~--~~ ~~--~~32.30±3.39	--	**<0.001**
δ^{15}N	0.~~177~~196±0.~~029~~ ~~11.4~~025	14.8	**<0.001**	δ^{13}C	1.164±0.~~882±~~115	34.0~~.145~~ ~~13.9~~	**<0.001**
%N	1.~~14~~036±0.~~18~~ ~~12.1~~162	9.5	**<0.001**	%N	-2.~~566~~963±0.~~406~~ ~~14.9~~360	22.5	**<0.001**
%C	-0.~~036~~033±0.011	2.3~~.1~~	**0.002**	%C	0.~~089~~107±0.~~025~~ ~~4.8~~023	7.0	**<0.001**
C/N	0.~~023~~025±0.004	11.~~5~~0	**<0.001**	C/N	-0.~~044~~060±0.~~009~~ ~~9.7~~008	17.1	**<0.001**
Hummock	0.44±0.07	9.6	**<0.001**	Hummock	--	--	--
Treed	-0.~~15~~12±0.~~06~~ ~~1.9~~05	3.5	**0.~~014~~023**	Treed	~~0.29±0.13~~--	--	--
Depth	-4.18±0.44e^{-2}	21.2	**<0.001**	Depth	-2.48±0.63e^{-2}	5.1	**<0.001**
Depth^2	-3.28±0.40e^{-4}	15.4	**<0.001**	Depth^2	-1.57±0.68e^{-4}	1.8	**0.~~029~~021**
~~Hummock~~	~~0.32±0.12~~	~~2.3~~	~~**0.007**~~	~~Hummock~~	~~0.71±0.27~~	~~2.7~~	~~**0.008**~~
~~Plot~~	~~--~~	~~10.0~~	~~**0.008**~~	~~Plot~~	~~--~~	~~14.6~~	~~**0.001**~~
~~Depth~~	~~--~~	~~47.6~~	~~**<0.001**~~	~~Depth~~	~~--~~	~~37~~	
Depth^3	-7.03±1.21e^{-7}	7.9	**<0.001**	Depth^3	-4.89±2.27e^{-7}	1.6	**0.032**
Group (1)	-0.121±0.031	3.5	**<0.001**	Group (5)	-0.305±0.070	6.3	**<0.001**
Group (2)	0.151±0.081	1.3	0.062	Group (6)	0.280±0.188	0.7	0.137
Group (3)	-0.111±0.047	1.5	**0.018**	Group (7)	-0.308±0.090	3.9	**<0.001**
Group (4)	-0.163±0.065	1.2	**0.013**				

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Table 4. Correlations between radiocarbon and stable isotopes by depth, and mean Δ^{14}C for that depth. Hummock vs hollow plots and treed vs non-treed plots were averaged together. δ^{13}C±se and δ^{15}N±se columns reflect the shift in Δ^{14}C with a 1‰ shift in δ^{13}C or δ^{15}N. Statistically significant correlations are bolded. nd = not determined.

Depth	n	Mean±se Δ^{14}C (‰)	Age (cal yr BP)	δ^{13}C±se adj. r^2	(‰)	P	δ^{15}N±se adj. r^2	(‰)	P
25	3	52±5	Modern	0.881	10±3	0.~~1572~~157	-0.943	2±9	0.~~8916~~892
22	7	29±13	Modern	0.325	69±35	0.~~1057~~106	-0.195	2±11	0.889
15	16	59±5	Modern	**0.219**	**13±6**	**0.~~0388~~039**	**0.221**	**7±3**	**0.038**
5	16	115±18	Modern	**0.682**	**74±13**	**<0.~~0001~~001**	0.039	17±14	0.~~2247~~225
-5	19	71±7	Modern	**0.352**	**33±10**	**0.~~0044~~004**	**0.293**	**18±6**	**0.~~0098~~010**
-15	19	126±19	Modern	-0.053	9±30	0.~~7619~~762	-0.024	-16±22	0.~~4591~~459
-25	20	100±23	Modern	0.018	-41±35	0.~~2613~~261	**0.414**	**-62±16**	**0.~~0013~~001**
-35	18	-102±34	1580±180	**0.228**	**-246±100**	**0.~~0261~~026**	-0.053	-9±24	0.~~7045~~704
-45	18	-182±27	1730±260	-0.041	-54±95	0.575	**0.570**	**58±12**	**<0.~~0002~~001**
-55	18	-203±19	nd	-0.025	-63±81	0.~~4474~~447	**0.512**	**52±12**	**<0.~~0007~~001**
-65	18	-288±11	2800±600	**0.486**	**-100±24**	**<0.~~0008~~001**	**0.340**	**44±14**	**0.~~0065~~006**
-85	18	-359±7	3520±90	-0.007	-23±25	0.~~3618~~362	-0.049	-7±15	0.~~6505~~651
-113	18	-392±6	3950±90	0.081	42±27	0.~~1337~~134	0.016	-17±15	0.~~2756~~276
-163	17	-486±7	6000±500	**0.713**	**68±11**	**<0.~~0001~~001**	**0.537**	**-31±7**	**<0.~~0005~~001**
-213	3	-587±32	9200±200	-0.811	-44±136	0.801	-0.96	-11±76	0.~~9115~~912
-225	11	-567±12	6775±260	-0.089	27±62	0.~~6771~~677	-0.101	9±33	0.~~7842~~784

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12 Figures

Figure 1. Conceptual diagram of movement of carbon (C) and nitrogen (N) during peatland development from rich fen to bog. Major processes influencing isotopic composition include methane (Me) flux from surficial and deeper layers (dotted upward arrow), methanotrophy and subsequent CO_2 recapture by *Sphagnum*, vascular plant transport of methane, N uptake by vascular plants and mycorrhizal fungi, and the sequestration of C and N over time in deeper peat. Assimilation of ^{13}C-depleted CO_2 from the Suess effect influences modern peat carbon (rightmost top box); N flux from adjacent uplands influences productivity in the lagg region, ~~and hummock and hollow~~peatland topography ~~within peatlands~~of hummocks and hollows influences methanogenesis and methanotrophy~~.~~, and trees influence partitioning of nitrogen. Climate (not shown) will influence the initial ~~δ~~δ^{13}C of fixed carbon. By rotating the figure 90° counterclockwise, the lower boxes correspond stratigraphically to the peat profile.

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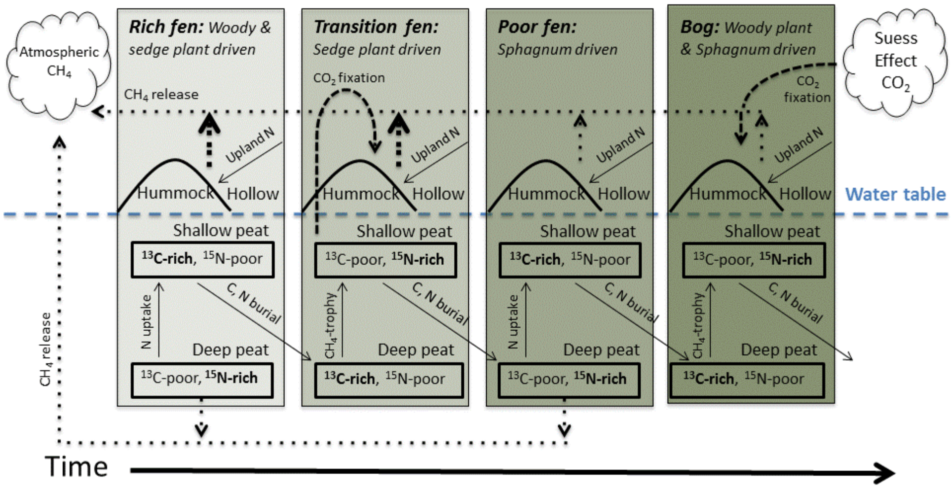

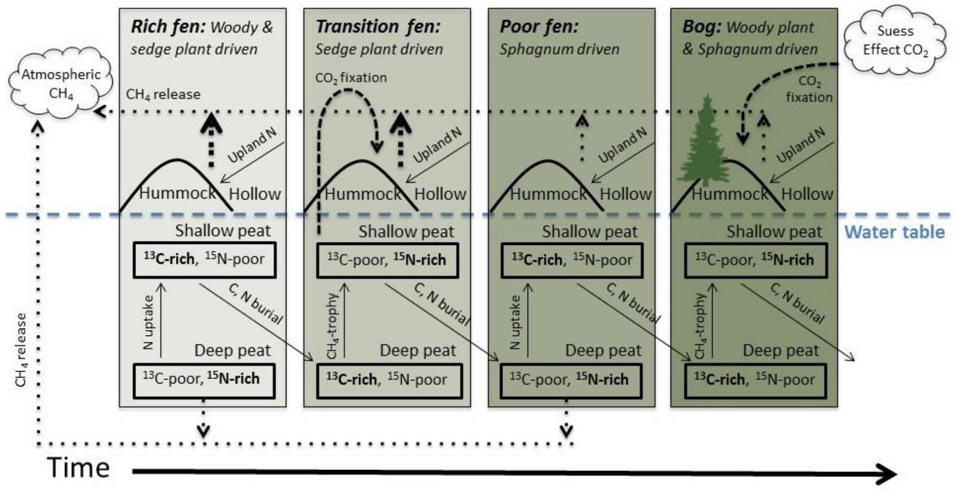
Rich fen: Woody & sedge plant driven
Transition fen: Sedge plant driven
Poor fen: Sphagnum driven
Bog: Woody plant & Sphagnum driven
Atmospheric CH$_4$
Suess Effect CO$_2$
CH$_4$ release
CO$_2$ fixation
CO$_2$ fixation
Upland N
Upland N
Upland N
Upland N
Hummock
Hollow
Hummock
Hollow
Hummock
Hollow
Hummock
Hollow
Water table
Shallow peat
Shallow peat
Shallow peat
Shallow peat
^{13}C-rich, ^{15}N-poor
^{13}C-poor, ^{15}N-rich
^{13}C-rich, ^{15}N-poor
^{13}C-poor, ^{15}N-rich
N uptake
CH$_4$-trophy
N uptake
CH$_4$-trophy
C, N burial
C, N burial
C, N burial
C, N burial
Deep peat
Deep peat
Deep peat
Deep peat
^{13}C-poor, ^{15}N-rich
^{13}C-rich, ^{15}N-poor
^{13}C-poor, ^{15}N-rich
^{13}C-rich, ^{15}N-poor
CH$_4$ release
Time

Figure 2. Aerial photograph of the S1 bog ~~(23 September 2014)~~ showing the 17 experimental plots (each 10.4 m in diameter to the outer edge of the visible perimeter boardwalk). Plot numbers on the image represent the plot areas within which peat was sampled. The lagg boundary is delineated with a dashed line, and the inset indicates the location of the bog within Minnesota and the Marcell Experimental Forest.

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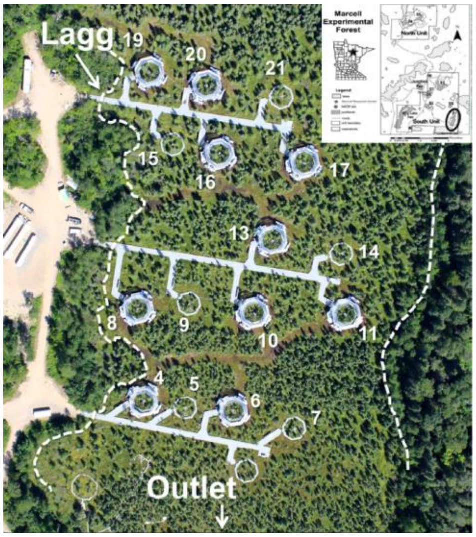

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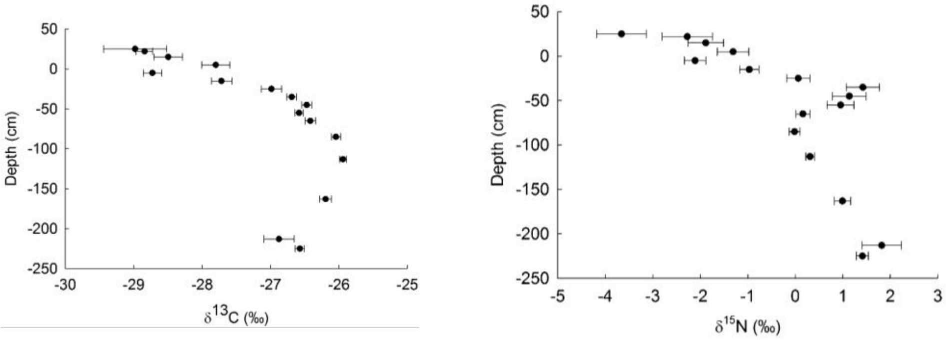
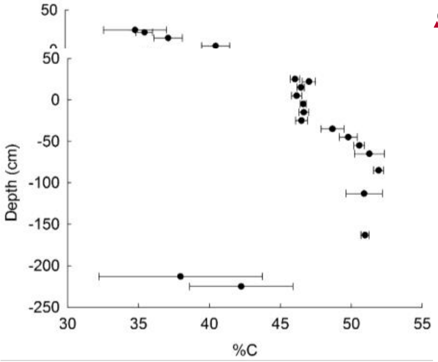
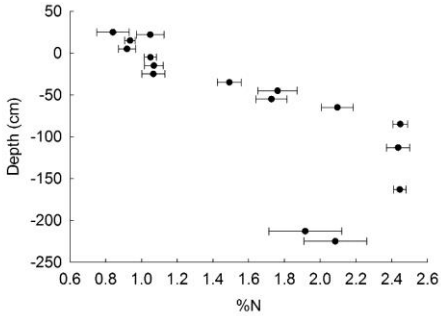
Figure 3. δ^{15}N, δ^{13}C, %N, %C, and C/N versus depth. Values (±se) are averaged across hummock versus hollow cores and across treed versus non-treed cores. Averages (±se) are given for each depth, with *n* given in parentheses following the depth: 25 cm (3), 22 cm (7), 15 cm (14), 5 cm (14), -5 cm (19), -15 cm (17), -25 cm (17), -35 cm (15), -45 cm (17), -55 cm (17), -65 cm (17), -85 cm (18), -112 cm (17), -162 cm (17), -213 cm (3), -225 cm (8).

a. δ^{13}C versus depth b. δ^{15}N versus depth



c. %C versus depth d. %N versus depth





e. C/N.

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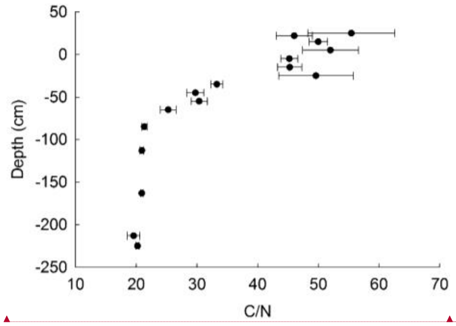

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Figure 4. Depth coefficients of δ^{15}N and δ^{13}C calculated from the three depth terms in the regression models~~correlate in peat profiles.~~. The depth in cm for specific points is indicated ~~next to coefficients (±se).~~on the line.

~~$\delta^{15}N = 1.97\pm0.44 \times \delta^{13}C + 0.00\pm0.34$, adjusted $r^2 = 0.557$, $n = 16$, $p = 0.0005$~~

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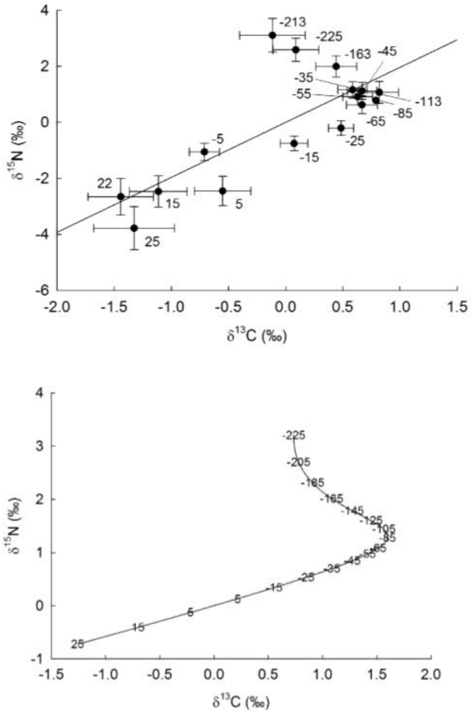

Figure 5. ~~a.~~ Plot coefficients of δ^{15}N and δ^{13}C from regression models ~~correlate~~ in peat profiles~~.~~ ~~Standard error bars omitted for clarity, and averaged 0.24‰ for δ^{15}N and 0.12‰ for δ^{13}C.~~, as calculated from Table 1. The plot number is the symbol for the paired coefficient values. ~~Data plotted below, δ^{15}N = –1.36±0.46 × δ^{13}C + 0.00±0.08, adjusted r^2 = 0.271, p = 0.0224, n = 16.~~ ~~b. To show the spatial relationship among coefficient~~For clarity, plots with identical or near-identical values~~, plot locations are as given in Figure 2, with plot 4 at lower left. Values are given × 10 for the δ^{15}N and δ^{13}C coefficients, as (δ^{15}N, δ^{13}C). Coefficients are color-coded based on δ^{15}N values,~~ indicated with ~~blue = high δ^{15}N, red = low δ^{15}N, and purple intermediate δ^{15}N.~~ circles.

~~a.~~ ~~b.~~

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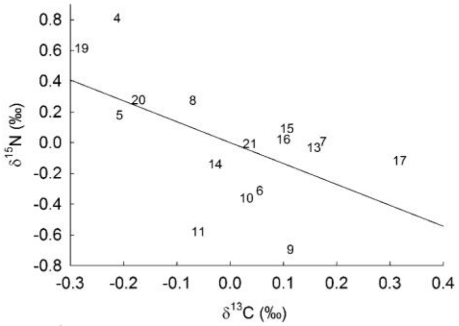

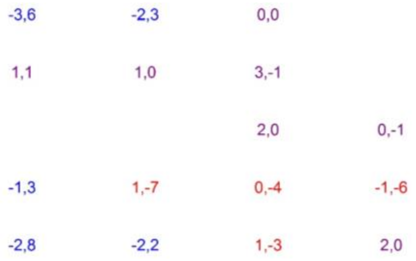

-3,6	-2,3	0,0	
1,1	1,0	3,-1	
		2,0	0,-1
-1,3	1,-7	0,-4	-1,-6
-2,8	-2,2	1,-3	2,0

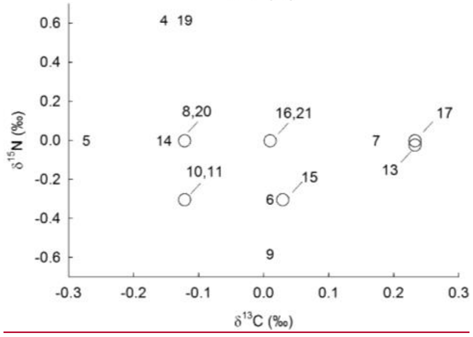

Figure 6. Relationship between $\Delta^{14}C$ and a) $\delta^{13}C$; b) $\delta^{15}N$.

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a. b.

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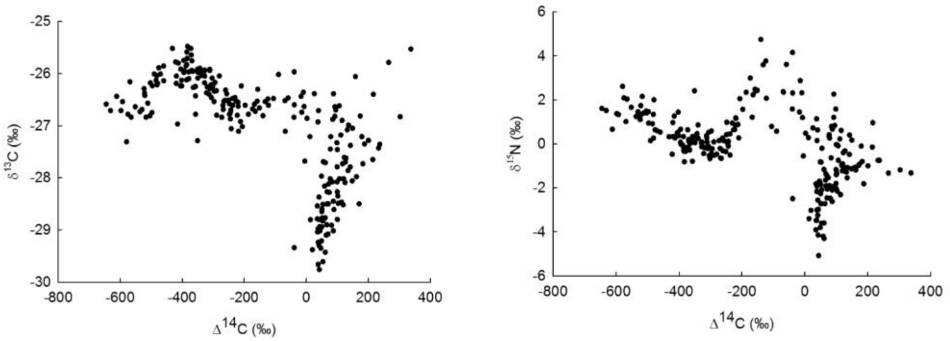

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13 Appendices

~~Table A1. Regression model for explaining δ^{13}C and δ^{15}N in peat profiles at SPRUCE. *Treed* vs non-treed, *hummock* vs. hollow topography. Plot and depth treated as nominal variables. Value = Coefficient ± standard error; Var. = % variance explained. n = 238.~~

~~δ^{13}C model, adjusted r^2 = 0.853, p <0.001~~				~~δ^{15}N model, adjusted r^2 = 0.701, p <0.001~~			
~~Source~~	~~Value±se~~	~~%Var.~~	~~P~~	~~Source~~	~~Value±se~~	~~%Var.~~	~~P~~
~~Intercept~~	~~-27.80±0.38~~	~~--~~	~~--~~	~~Intercept~~	~~25.21±4.09~~	~~--~~	~~--~~
~~δ^{15}N~~	~~0.177±0.029~~	~~11.4~~	~~**<0.001**~~	~~δ^{13}C~~	~~0.882±0.145~~	~~13.9~~	~~**<0.001**~~
~~%N~~	~~1.14±0.18~~	~~12.1~~	~~**<0.001**~~	~~%N~~	~~-2.566±0.406~~	~~14.9~~	~~**<0.001**~~
~~%C~~	~~-0.036±0.011~~	~~3.1~~	~~**0.002**~~	~~%C~~	~~0.089±0.025~~	~~4.8~~	~~**<0.001**~~
~~C/N~~	~~0.023±0.004~~	~~11.5~~	~~**<0.001**~~	~~C/N~~	~~-0.044±0.009~~	~~9.7~~	~~**<0.001**~~
~~Treed~~	~~-0.15±0.06~~	~~1.9~~	~~**0.014**~~	~~Treed~~	~~0.29±0.13~~	~~1.8~~	~~**0.029**~~
~~Hummock~~	~~0.32±0.12~~	~~2.3~~	~~**0.007**~~	~~Hummock~~	~~0.71±0.27~~	~~2.7~~	~~**0.008**~~
~~Plot~~	~~--~~	~~10.0~~	~~**0.008**~~	~~Plot~~	~~--~~	~~14.6~~	~~**0.001**~~
~~Depth~~	~~--~~	~~47.6~~	~~**<0.001**~~	~~Depth~~	~~--~~	~~37.5~~	~~**<0.001**~~
~~Plot~~				~~Plot~~			
~~4~~	~~-0.22±0.09~~		~~**0.0241**~~	~~4~~	~~0.81±0.21~~		~~**0.0001**~~
~~5~~	~~-0.21±0.10~~		~~**0.0327**~~	~~5~~	~~0.18±0.22~~		~~0.4244~~
~~6~~	~~0.05±0.11~~		~~0.6733~~	~~6~~	~~-0.31±0.24~~		~~0.1933~~
~~7~~	~~0.17±0.11~~		~~0.1042~~	~~7~~	~~0.00±0.24~~		~~0.9875~~
~~8~~	~~-0.07±0.11~~		~~0.5042~~	~~8~~	~~0.27±0.24~~		~~0.2656~~
~~9~~	~~0.11±0.11~~		~~0.3187~~	~~9~~	~~-0.70±0.24~~		~~**0.0043**~~
~~10~~	~~0.03±0.11~~		~~0.8007~~	~~10~~	~~-0.36±0.24~~		~~0.1295~~
~~11~~	~~-0.06±0.11~~		~~0.5791~~	~~11~~	~~-0.58±0.25~~		~~**0.0208**~~
~~13~~	~~0.15±0.11~~		~~0.1569~~	~~13~~	~~-0.03±0.24~~		~~0.8883~~
~~14~~	~~-0.03±0.11~~		~~0.7861~~	~~14~~	~~-0.14±0.26~~		~~0.5796~~
~~15~~	~~0.10±0.12~~		~~0.3708~~	~~15~~	~~0.09±0.26~~		~~0.7274~~
~~16~~	~~0.10±0.11~~		~~0.3602~~	~~16~~	~~0.02±0.24~~		~~0.9334~~
~~17~~	~~0.32±0.11~~		~~**0.0033**~~	~~17~~	~~-0.12±0.24~~		~~0.6275~~
~~19~~	~~-0.28±0.11~~		~~**0.0088**~~	~~19~~	~~0.61±0.24~~		~~**0.011**~~
~~20~~	~~-0.17±0.11~~		~~0.1072~~	~~20~~	~~0.28±0.24~~		~~0.2562~~
~~21~~	~~0.03~~		~~--~~	~~21~~	~~-0.01~~		~~--~~
~~Depth~~							
~~25~~	~~-1.33±0.35~~		~~**0.0002**~~	~~25~~	~~-3.78±0.77~~		~~**<0.0001**~~
~~22~~	~~-1.44±0.29~~		~~**<0.0001**~~	~~22~~	~~-2.65±0.65~~		~~**<0.0001**~~
~~15~~	~~-1.11±0.25~~		~~**<0.0001**~~	~~15~~	~~-2.47±0.56~~		~~**<0.0001**~~
~~5~~	~~-0.55±0.25~~		~~**0.0266**~~	~~5~~	~~-2.45±0.53~~		~~**<0.0001**~~
~~-5~~	~~-0.71±0.13~~		~~**<0.0001**~~	~~-5~~	~~-1.06±0.31~~		~~**0.0007**~~
~~-15~~	~~0.07±0.12~~		~~0.5328~~	~~-15~~	~~-0.76±0.26~~		~~**0.0043**~~
~~-25~~	~~0.49±0.11~~		~~**<0.0001**~~	~~-25~~	~~-0.20±0.26~~		~~0.4302~~
~~-35~~	~~0.59±0.13~~		~~**<0.0001**~~	~~-35~~	~~1.16±0.29~~		~~**<0.0001**~~
~~-45~~	~~0.67±0.13~~		~~**<0.0001**~~	~~-45~~	~~1.12±0.30~~		~~**0.0002**~~
~~-55~~	~~0.63±0.13~~		~~**<0.0001**~~	~~-55~~	~~0.92±0.30~~		~~**0.0024**~~
~~-65~~	~~0.67±0.14~~		~~**<0.0001**~~	~~-65~~	~~0.63±0.32~~		~~0.0502~~
~~-85~~	~~0.79±0.16~~		~~**<0.0001**~~	~~-85~~	~~0.79±0.38~~		~~**0.0402**~~
~~-113~~	~~0.82±0.17~~		~~**<0.0001**~~	~~-113~~	~~1.07±0.39~~		~~**0.0068**~~
~~-163~~	~~0.44±0.18~~		~~**0.0145**~~	~~-163~~	~~2.00±0.38~~		~~**<0.0001**~~
~~-225~~	~~0.09±0.20~~		~~0.6549~~	~~-225~~	~~2.59±0.41~~		~~**<0.0001**~~

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~~-213~~	~~-0.11~~	~~--~~	~~-213~~	~~3.11~~	~~--~~

~~Table A2. Multiple regression of sample C/N as affected by the presence of trees, topography (hummock or hollow), plot #, depth, or lab notes. Lab notes indicated whether mineral material, woody debris, or other unusual material was present. Adjusted r^2 is 0.632, n = 238, p < 0.0001. Depth (in cm) corresponds to that used in regression analyses of $\delta^{15}N$ and $\delta^{13}C$ in Appendix 1. Depths are indicated in centimeters.~~

~~Source~~	~~%Variance~~	~~Sum of Squares~~	~~P~~
~~Vegetation~~	~~0.04~~	~~11~~	~~0.7309~~
~~Topography~~	~~1.48~~	~~374~~	~~**0.0453**~~
~~Lab notes (4)~~	~~12.89~~	~~3247~~	~~**<0.0001**~~
~~Plot (15)~~	~~10.62~~	~~2676~~	~~**0.0217**~~
~~Depth (15)~~	~~74.96~~	~~18885~~	~~**<0.0001**~~

~~Parameter Estimates~~			~~Parameter Estimates (continued)~~		
~~Term~~	~~Estimate±se~~	~~Prob>|t|~~	~~Term~~	~~Estimate±se~~	~~Prob>|t|~~
~~Intercept~~	~~36.17±3.27~~	~~**<0.0001**~~	~~Depth [25]~~	~~30.12±6.73~~	~~**<0.0001**~~
~~Trees[no trees]~~	~~0.49±1.41~~	~~0.7309~~	~~Depth [22]~~	~~19.83±5.39~~	~~**0.0003**~~
~~Topography[Hollow]~~	~~5.37±2.67~~	~~**0.0453**~~	~~Depth [15]~~	~~19.76±4.63~~	~~**<0.0001**~~
~~Lab Notes~~					
~~[Large root]~~	~~-5.21±8.51~~	~~0.5416~~	~~Depth [5]~~	~~23.45±4.69~~	~~**<0.0001**~~
~~[Mineral matter]~~	~~-4.19±4.91~~	~~0.3935~~	~~Depth [-5]~~	~~8.01±2.51~~	~~**0.0016**~~
~~[Mineral soil]~~	~~-6.10±6.29~~	~~0.3332~~	~~Depth [-15]~~	~~6.82±2.50~~	~~**0.007**~~
~~[normal]~~	~~-4.19±2.97~~	~~0.1604~~	~~Depth [-25]~~	~~9.15±2.41~~	~~**0.0002**~~
~~(Woody debris)~~	~~19.69~~	~~--~~	~~Depth [-35]~~	~~-4.45±2.67~~	~~0.0971~~
~~Plot[4]~~	~~-1.94±2.21~~	~~0.3815~~	~~Depth [-45]~~	~~-8.00±2.67~~	~~**0.0031**~~
~~Plot[5]~~	~~1.80±2.33~~	~~0.4404~~	~~Depth [-55]~~	~~-7.26±2.72~~	~~**0.0081**~~
~~Plot[6]~~	~~6.04±2.51~~	~~**0.0171**~~	~~Depth [-65]~~	~~-12.37±2.69~~	~~**<0.0001**~~
~~Plot[7]~~	~~2.09±2.51~~	~~0.4055~~	~~Depth [-85]~~	~~-16.40±2.67~~	~~**<0.0001**~~
~~Plot[8]~~	~~-1.50±2.62~~	~~0.5669~~	~~Depth [-113]~~	~~-16.80±2.67~~	~~**<0.0001**~~
~~Plot[9]~~	~~3.09±2.53~~	~~0.2242~~	~~Depth [-163]~~	~~-17.05±2.72~~	~~**<0.0001**~~
~~Plot[10]~~	~~-1.84±2.55~~	~~0.4714~~	~~Depth (-213)~~	~~-16.33~~	~~--~~
~~Plot[11]~~	~~7.07±2.60~~	~~**0.0071**~~	~~Depth [-225]~~	~~-18.47±3.81~~	~~**<0.0001**~~
~~Plot[13]~~	~~-7.23±2.55~~	~~**0.0049**~~			
~~Plot[14]~~	~~-1.28±2.73~~	~~0.6398~~			
~~Plot[15]~~	~~-3.81±2.81~~	~~0.1765~~			
~~Plot[16]~~	~~2.02±2.53~~	~~0.4251~~			
~~Plot[17]~~	~~1.23±2.51~~	~~0.6234~~			
~~Plot[19]~~	~~-2.73±2.62~~	~~0.2985~~			
~~Plot[20]~~	~~-0.86±2.60~~	~~0.7409~~			
~~Plot 21~~	~~1.90~~	~~--~~			

~~Least Squares Means Table for C/N of Categories from Lab Notes~~

~~Level~~	~~Least Squares Mean±se~~	~~Mean~~
~~Large root~~	~~30.97±10.40~~	~~46.8~~
~~Mineral matter~~	~~31.98±5.48~~	~~20.02~~
~~Mineral soil~~	~~30.07±7.53~~	~~19.55~~
~~normal~~	~~31.99±1.80~~	~~34.3555~~
~~Woody debris~~	~~55.86±4.37~~	~~69.4143~~

~~Table A3. Correlation of %N with δ^{15}N by depth. Significant P values are bolded.~~

~~Depth (cm)~~	~~%N coefficent±se~~	~~P~~	~~intercept±se~~	~~P~~
~~25~~	~~1.68±5.42~~	~~0.8089~~	~~-5.07±4.61~~	~~0.4697~~
~~22~~	~~4.21±2.48~~	~~0.1506~~	~~-6.69±2.64~~	~~0.0525~~
~~15~~	~~3.88±3.17~~	~~0.2408~~	~~-5.52±2.99~~	~~0.0859~~
~~5~~	~~3.13±1.62~~	~~0.0732~~	~~-4.19±1.52~~	~~**0.0153**~~
~~-5~~	~~4.62±1.11~~	~~**0.0006**~~	~~-6.96±1.17~~	~~**<0.0001**~~
~~-15~~	~~2.58±0.71~~	~~**0.0022**~~	~~-3.73±0.78~~	~~**0.0002**~~
~~-25~~	~~2.73±0.66~~	~~**0.0006**~~	~~-2.84±0.73~~	~~**0.001**~~
~~-35~~	~~-1.66±1.27~~	~~0.2093~~	~~3.90±1.93~~	~~0.0598~~
~~-45~~	~~-2.45±0.53~~	~~**0.0003**~~	~~5.45±0.97~~	~~**<0.0001**~~
~~-55~~	~~-2.70±0.45~~	~~**<0.0001**~~	~~5.61±0.80~~	~~**<0.0001**~~
~~-65~~	~~-0.72±0.38~~	~~0.0719~~	~~1.68±0.80~~	~~0.052~~
~~-85~~	~~0.02±0.70~~	~~0.9788~~	~~-0.06±1.71~~	~~0.9716~~
~~-113~~	~~-0.21±0.36~~	~~0.5716~~	~~0.82±0.89~~	~~0.3686~~
~~-163~~	~~-2.21±1.14~~	~~0.0724~~	~~6.39±2.80~~	~~**0.0373**~~
~~-213~~	~~-1.99±0.27~~	~~0.0859~~	~~5.63±0.52~~	~~0.059~~
~~-225~~	~~0.01±0.24~~	~~0.9821~~	~~1.40±0.52~~	~~**0.0237**~~

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